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Metabolic Investigations of Molecular Mechanisms Associated with Parkinson's Disease.

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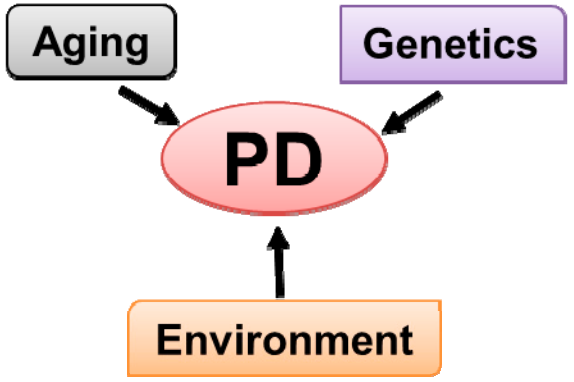
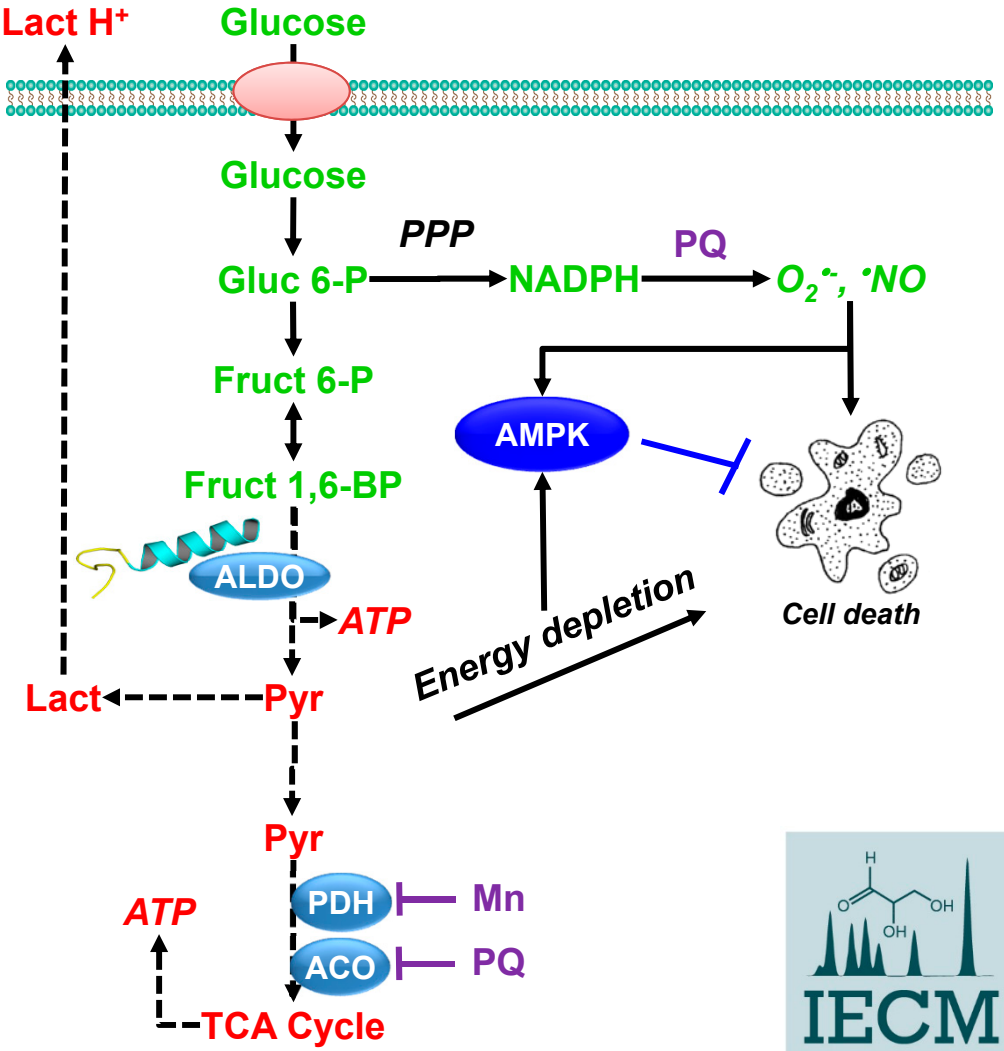
Metabolic Investigations of Molecular Mechanisms Associated with Parkinson's Disease

Graphical Abstract





IECM

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by fibrillar cytoplasmic aggregates of α-synuclein (i.e., Lewy bodies [LB]) and the associated loss of dopaminergic cells in the *substantia nigra.* But, mutations in genes such as α-synuclein (SNCA) account for only 10% of PD occurrences. The exposure to environmental toxicants including pesticides (e.g. paraquat [PQ]) and manganese (Mn), are also recognized as important PD risk factors. Thus, aging, genetic alterations and environmental factors all contribute to the etiology of PD. In fact, both genetic and environmental factors are thought to interact in the promotion of idiopathic PD, but the mechanisms involved are still unclear. In this study, we report a toxic synergistic effect between α-synuclein and either paraquat or Mn treatment. We identified an essential role for central carbon (glucose) metabolism in dopaminergic cell death induced by paraquat or Mn treatment that is enhanced by the overexpression of α-synuclein. PQ "hijacks" the pentose phosphate pathway (PPP) to increase NADPH reducing equivalents and stimulate paraquat redox cycling, oxidative stress, and cell death. PQ also stimulated an increase in glucose uptake, the translocation of glucose transporters to the plasma membrane, and AMPK activation. The overexpression of α-synuclein further stimulated an increase in glucose uptake and AMPK activity, but impaired glucose metabolism. In effect, α-synuclein activity directs additional carbon to the PPP to supply paraquat redox cycling. Alternatively, Mn induces an upregulation in glycolysis and the malate-aspartate shuttle to compensate for energy depletion due to Mn toxicity. Mn treatment causes a decrease in carbon flow through the TCA cycle and a disruption in pyruvate metabolism, which are consistent with a dysfunctional mitochondria and inhibition of pyruvate dehydrogenase. The overexpression of α-synuclein was shown to potentiate Mn toxicity by glycolysis impairment by inhibiting aldolase activity. In effect, α-synuclein overexpression negates the metabolic response to alleviate Mn toxicity that results in an increase in cell death.

Keywords: Parkinson's Disease; genetics-toxin synergy; molecular mechansims; NMR & MS

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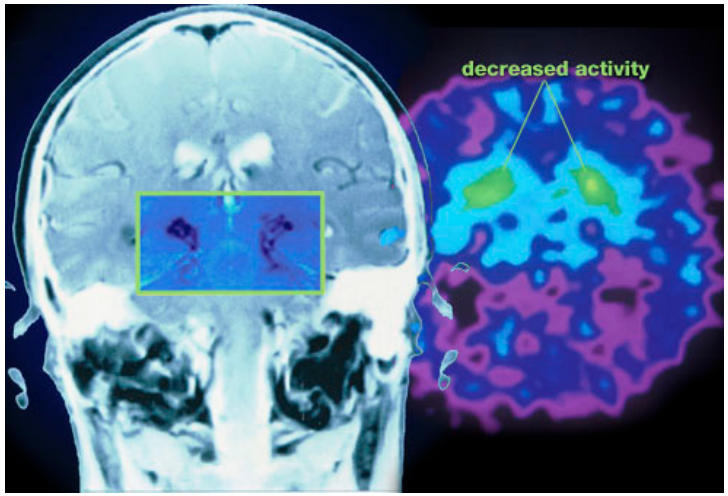
Introduction – Seminar Outline

- Overview of Parkinson's disease (PD).
- Combining NMR and MS in metabolomics
- Results of Paraquat and Manganese Treatment of Dopaminergic Neuronal Cells.
- Synergistic Effect of α-synuclein Overexpression and Paraquat/Manganese Treatment
- Conclusion



http://www.webmd.com/parkinsons-disease/ss/slideshow-parkinsons-overview

Nebraska	Age (years)			
	60-70	70-80	80+	≥60
PD Prevalence *(per 100,000)*	350	1,321	2,575	*1,183*

Estimated US Prevalence: 916,348 (≥40 y.o.)

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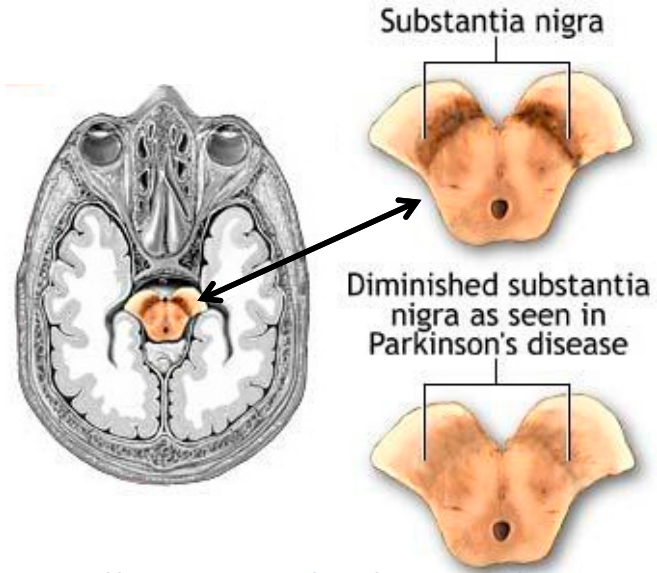
Introduction - Parkinson's disease (PD)

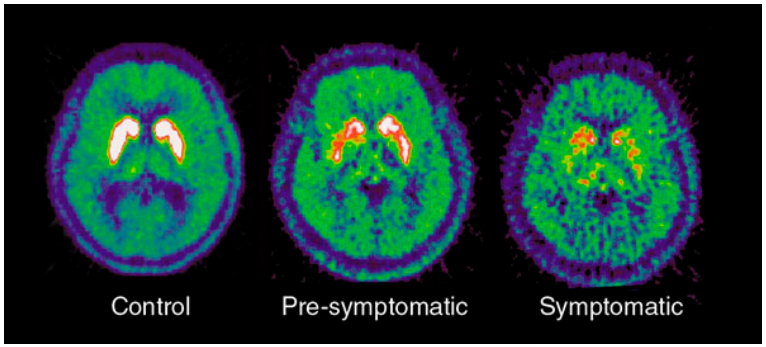
- Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder that leads to shaking (tremors) and difficulty with walking, movement, and coordination.

- Loss of dopaminergic neurons from the *substantia nigra* pars compacta leads to deficiency of dopamine in the caudate and putamen ("striatum").

- Currently, there is no cure for PD or a treatment to stop PD progression.



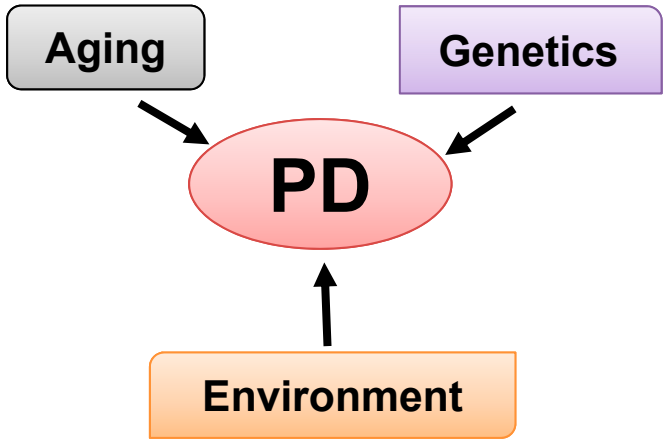
https://medlineplus.gov/ency/imagepages/19515.htm



Nature 399, A32-A39(24 June 1999)

Introduction – Causes of Parkinson's disease

- The exact cause of PD is unknown.
- Only 10% of PD is Familial (Hereditary).
  - Genetic alterations in *α-synuclein*, Parkin, DJ-1, PINK1 and LRRK2 have been associated with PD
- Sporadic (Idiopathic) PD are linked to genetic alterations, *environmental* or occupational factors
- Environmental agents linked to increased incidence/risk to develop Parkinson's disease
  - *Pesticides (paraquat)*
  - *Heavy Metals (manganese)*
  - Infectious agents
  - Industrialization
  - Dietary factors



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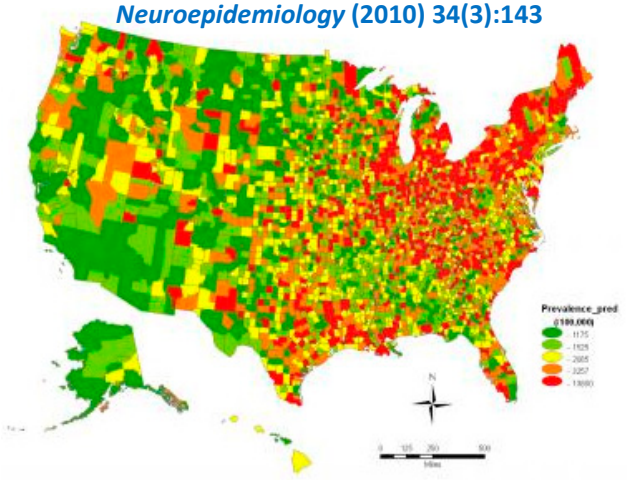
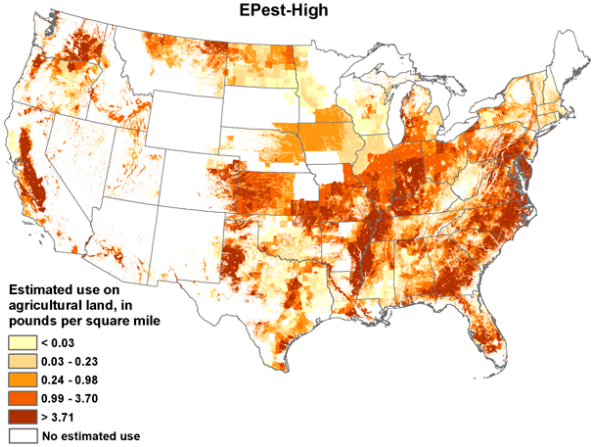
Introduction – Paraquat and Manganese are Environmental Risk Factors for PD

- Largest epidemiology study of Parkinson's disease in the US:
  - More common in Midwest and Northeast
  - Areas associated with Agriculture and Metal processing
- Environmental factors are likely common contributors to PD
  - Prolong exposure to herbicides and insecticides used in farming
  - Prolong exposure to metals, such as manganese
- Correlation between Paraquat agricultural usage and PD rates
- Paraquat selectively induces dopaminergic degeneration, one of the pathological hallmarks of PD.

Prevalence of Parkinson's disease in U.S.A
Neuroepidemiology (2010) 34(3):143



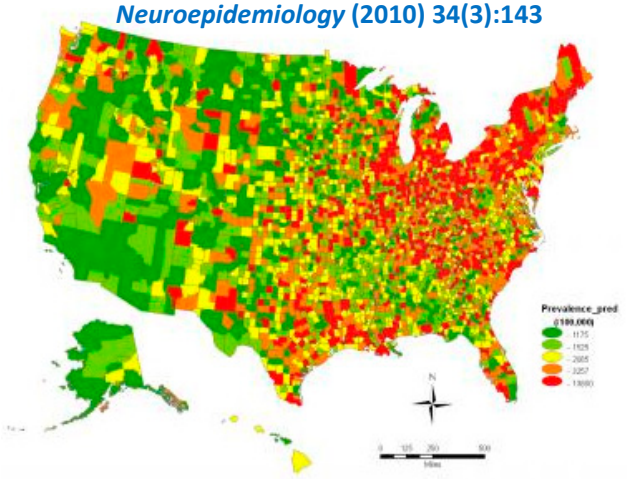
Estimated Agricultural Use for Paraquat , 2011
EPest-High



Introduction – Paraquat and Manganese are Environmental Risk Factors for PD

- Urban areas of East and Midwest contain the majority of metal-emitting facilities
  - PD more common in Midwest and Northeast
  - Mn 5th most abundant metal in the earth's crust
  - Mn essential cofactor for several enzymes (e.g., superoxide dismutase, SOD)
- Mn is environmental factors for idiopathic PD
  - "manganese-induced parkonsonism" or "manganism" similar symptoms with idiopathic PD.
  - Mn reported to specifically target dopaminergic neurons in *C. elegans* to cause neurodegeneration

Prevalence of Parkinson's disease in U.S.A
Neuroepidemiology (2010) 34(3):143



Annual Incidence of Parkinson's Disease in Urban Counties

Exposure Category	Incidence	95% Confidence Interval	Relative Risk	95% Confidence Interval
No reported copper, manganese, or lead[a]	274.0	226.8, 353.5		
High reported copper release	304.2	276.0, 336.8	1.11	0.94, 1.31
High reported manganese release	489.4	368.3, 689.5	1.78	1.54, 2.07
High reported lead release	285.7	249.3, 337.5	1.04	0.88, 1.23

[a]Less than 100 pounds (45.36 kg) of reported copper, manganese, or lead release.

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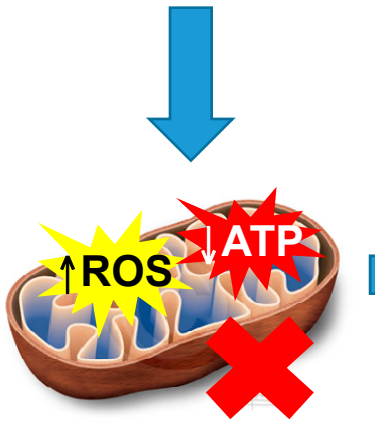
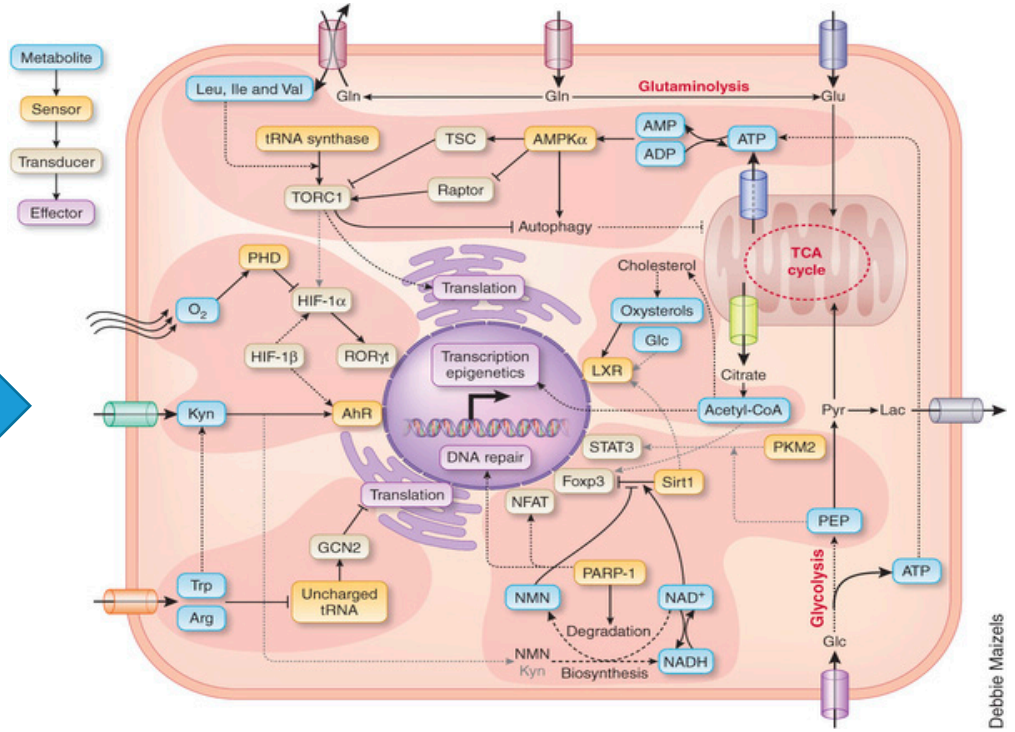
Introduction – Environmental Toxins & Mitochondrial Dysfunction

- Neurons have very high energy demands and high glucose usage
- Energy metabolism alterations have been reported in early PD
  - Mitochondrial dysfunction in PD
  - Toxins alters redox homeostasis, energy metabolism and central carbon metabolism
- A clear role for metabolomics in investigating PD

Environmental Toxicants



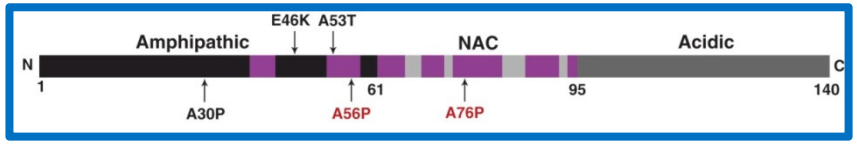


Debbie Maizels

IECM

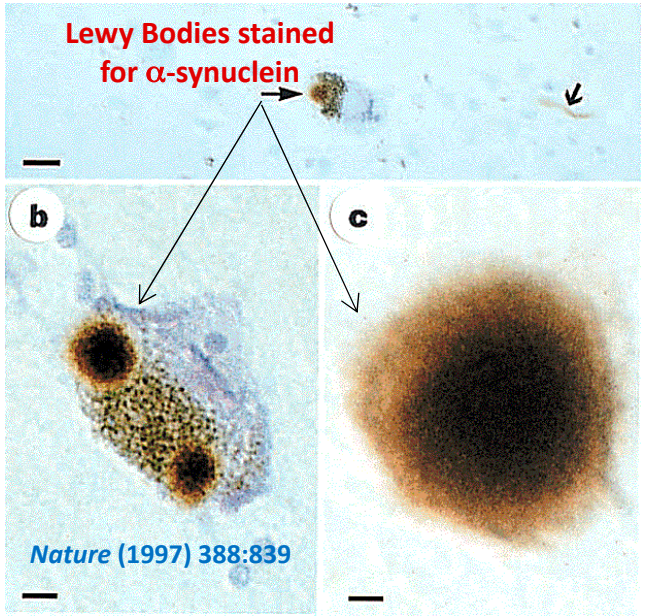
Introduction – α-Synuclein is a Genetic Risk Factor for PD

- Formation of intracellular aggregates (Lewy bodies) is a pathological hallmark of PD
- α-synuclein is a major component of Lewy Bodies
  - 140 aa soluble protein of unknown function

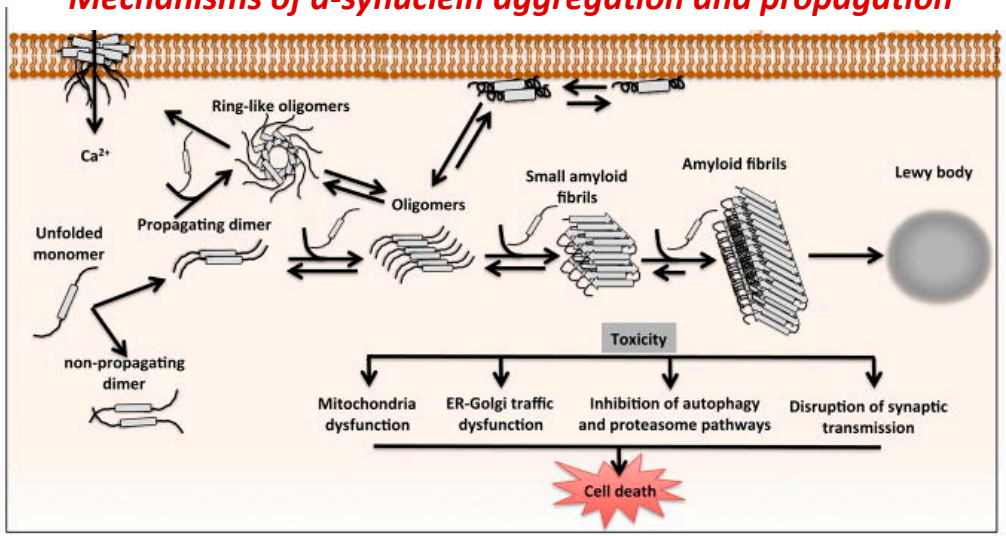


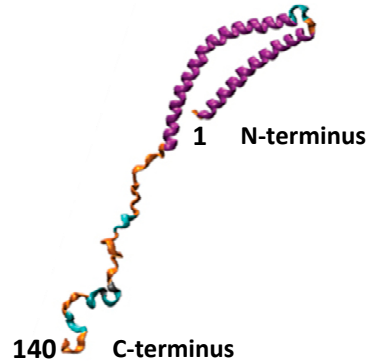
- Oligomerization of α-synuclein fibril formation is central to pathogenesis of PD

Substantia nigra from patients with PD



Mechanisms of a-synuclein aggregation and propagation





N at. Rev. Neurosci (2013) 14:38

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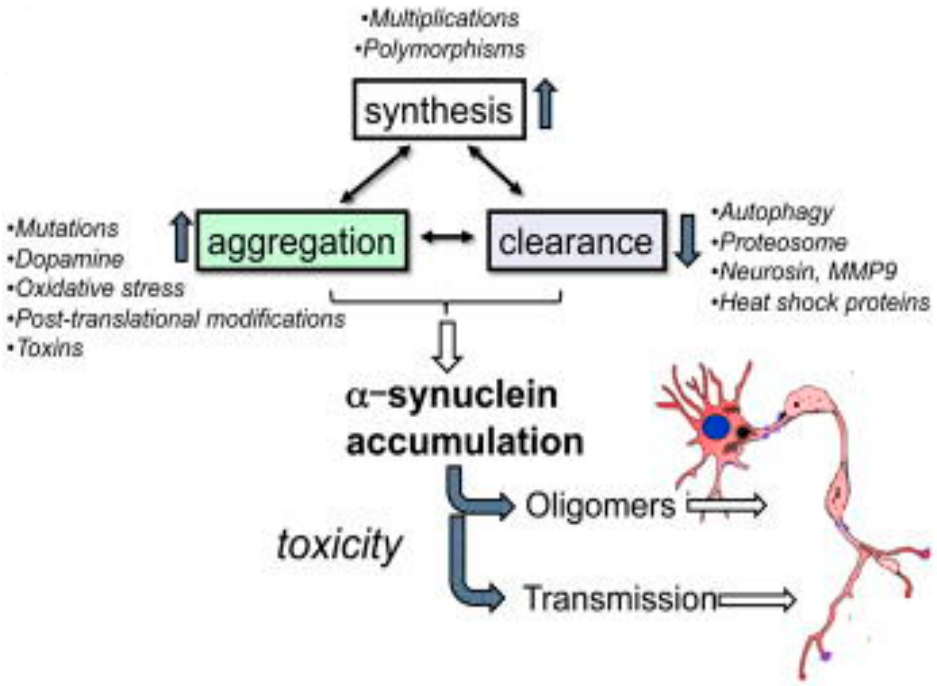
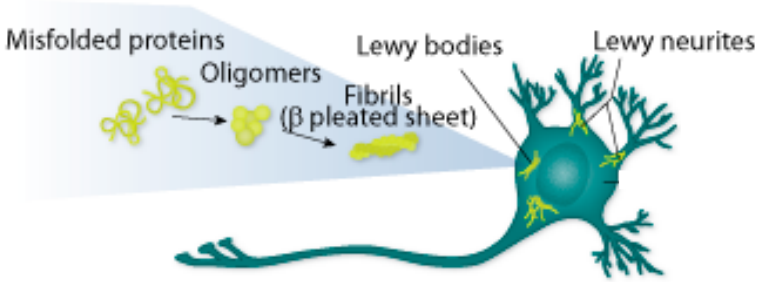
Introduction – Gene-Environment Interactions in PD

- **Mitochondrial dysfunction and energy failure induced by environmental toxicants can lead to α-synuclein misfolding and aggregation by an impairment in protein quality control mechanisms**





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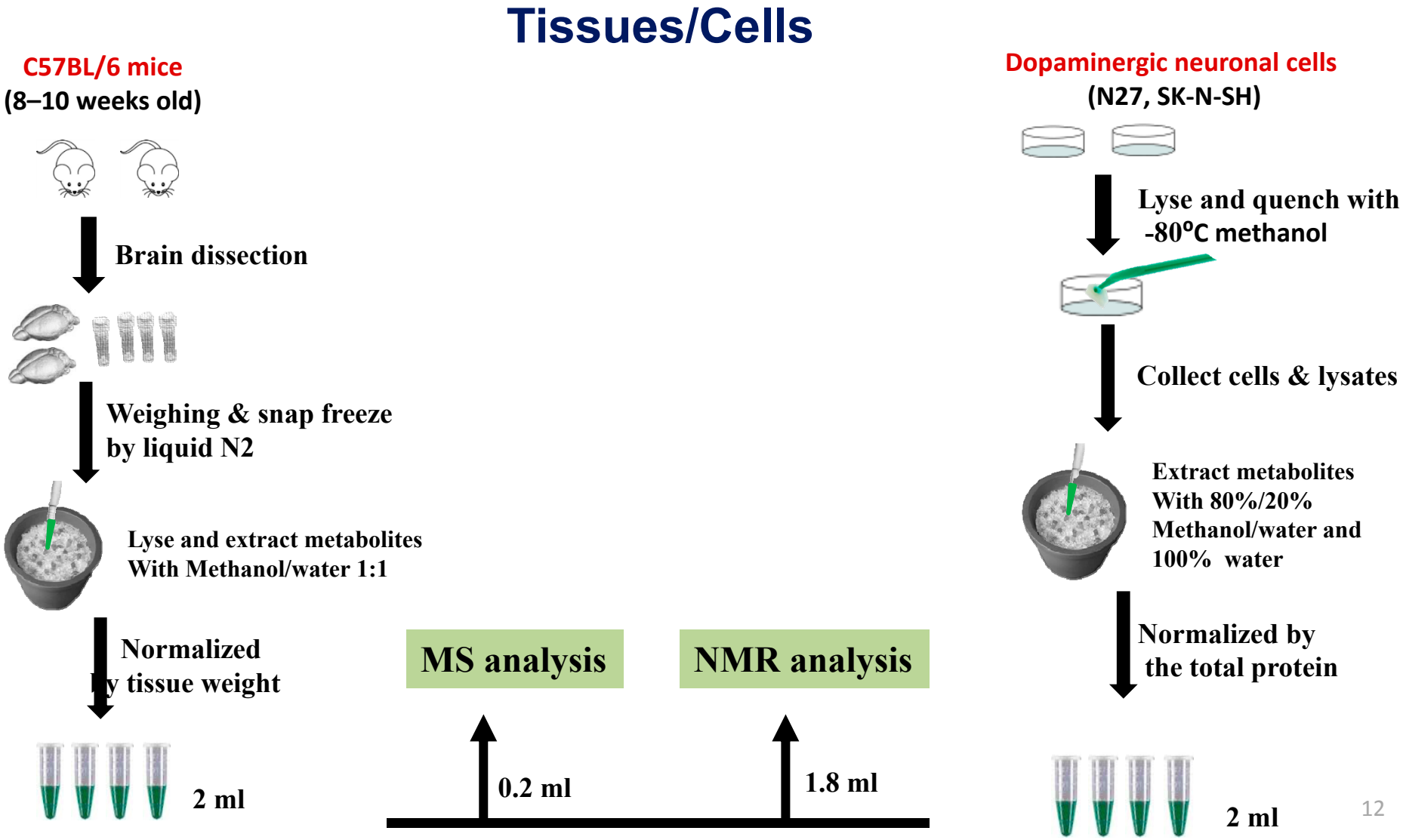
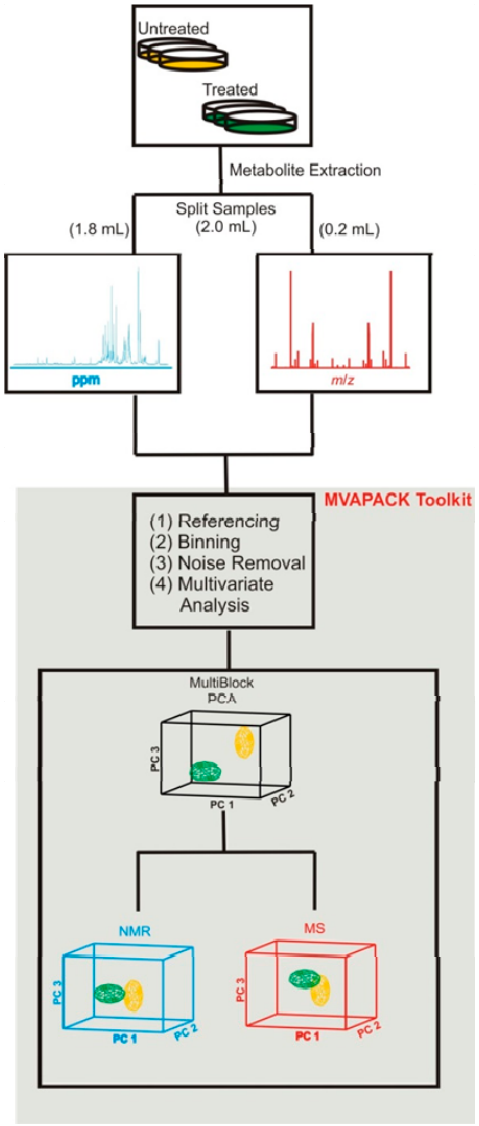
Results and discussion – Metabolomes Extracted from Dopaminergic Cells and Brain Tissues



Results and discussion – A Combined NMR and MS Metabolomics Protocol was applied to Investigate PD



Grow treated and untreated cells (10x)
- Extract metabolome
- Split for MS and NMR

Collect NMR and MS spectra
- NMR and MS spectra for each cell culture

Process NMR and MS spectra
- using our MVAPACK software

Generate Combined and Individual Model
- MB-PCA and MB-PLS
- using our MVAPACK software

Worley & Powers (2014) *ACS Chem. Biol.* 9(5):1138-1144.
Lei *et al.* (2014) *ACS Chem Biol.* 9(9):2032-2048
Marshall *et al.* (2015) *Metabolomics*, 11(2):391-402

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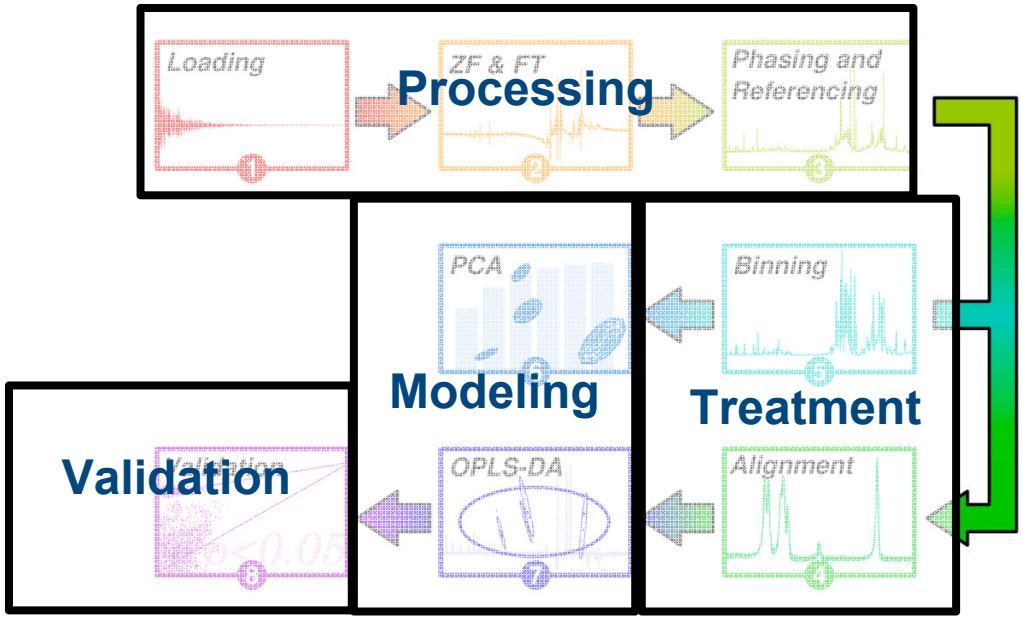
Results and discussion – NMR and MS Spectral Data Processed with Multiblock-PCA and our MVAPACK Software

Integrate Data From Multiple Analytical Methods

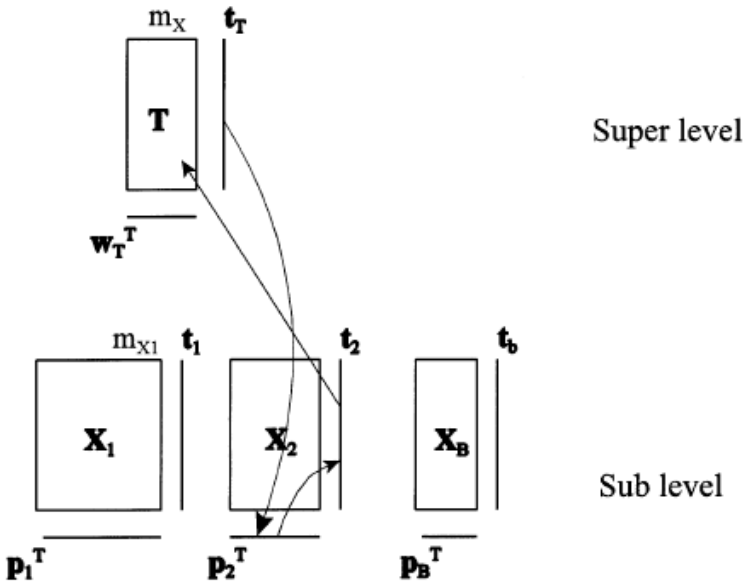
MVAPACK Metabolomics Toolkit

http://bionmr.unl.edu/mvapack.php



Worley & Powers (2014) *ACS Chem. Biol.* 9(5):1138-1144

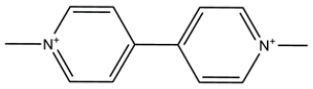
Multiblock-PCA

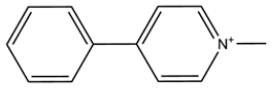


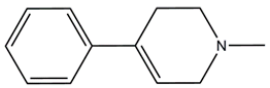
J. Chemometrics (1998) 12, 301–321

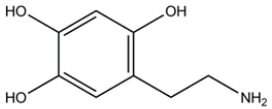
Results and discussion – Parkinson's Disease and Mitochondrial/Environmental Toxins

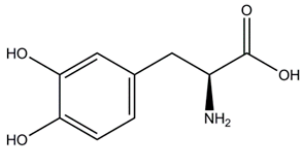
- **Mitochondrial dysfunction and energy failure Herbicides, pesticides, and designer drugs induce Parkinson's-like symptoms**
  - **Used as Equivalent molecular models for Parkinson's Disease**
  - **All result in dopaminergic neuronal cell death**
- **Our Metabolomics data indicate different molecular mechanisms of action**
  - **Focused on Paraquat (PQ)**

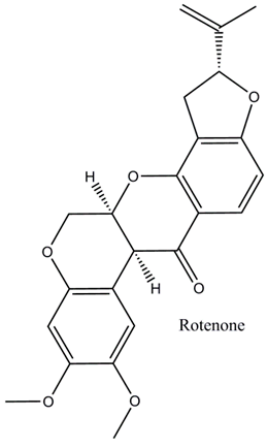

1,1'-Dimethyl-4,4'-bipyridinium (paraquat)

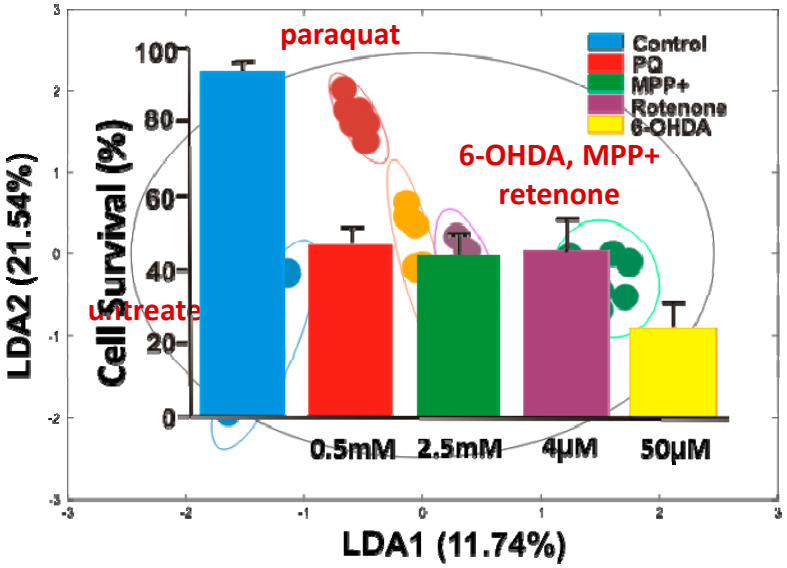

1-methyl-4-phenylpyridinium (MPP+)


1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)


6-hydroxydopamine (6-OHDA)


L-DOPA

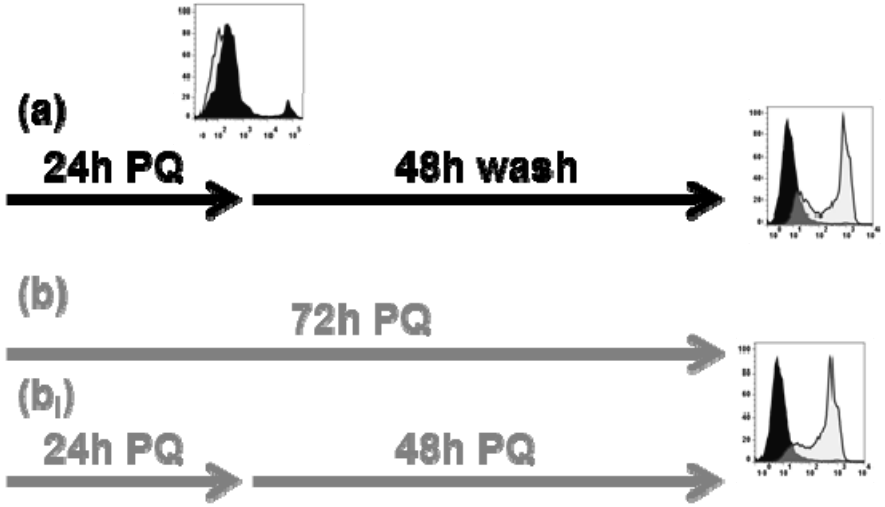

Rotenone



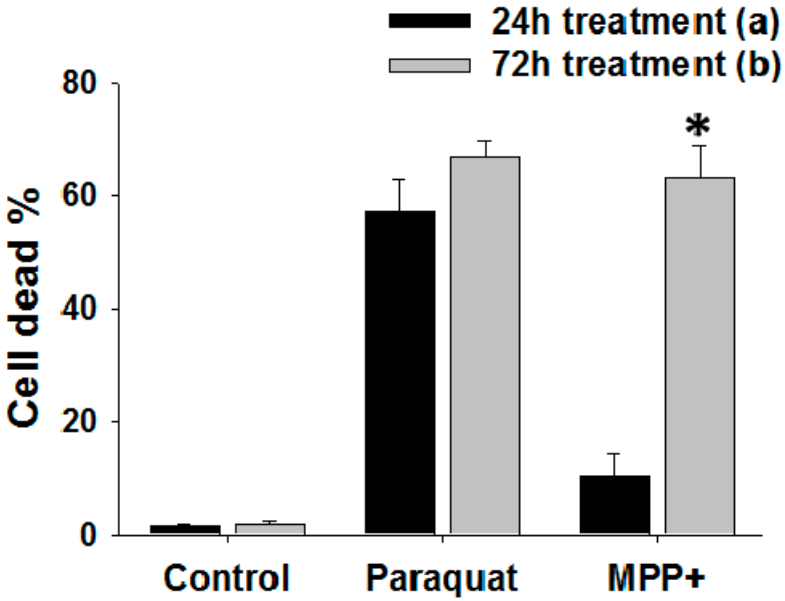
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

Results and discussion – Paraquat (PQ) Treatment of Dopaminergic Neuronal Cells Leads to Irreversible Cell Death

PQ Induces Irreversible Cell Death after 24 hrs.



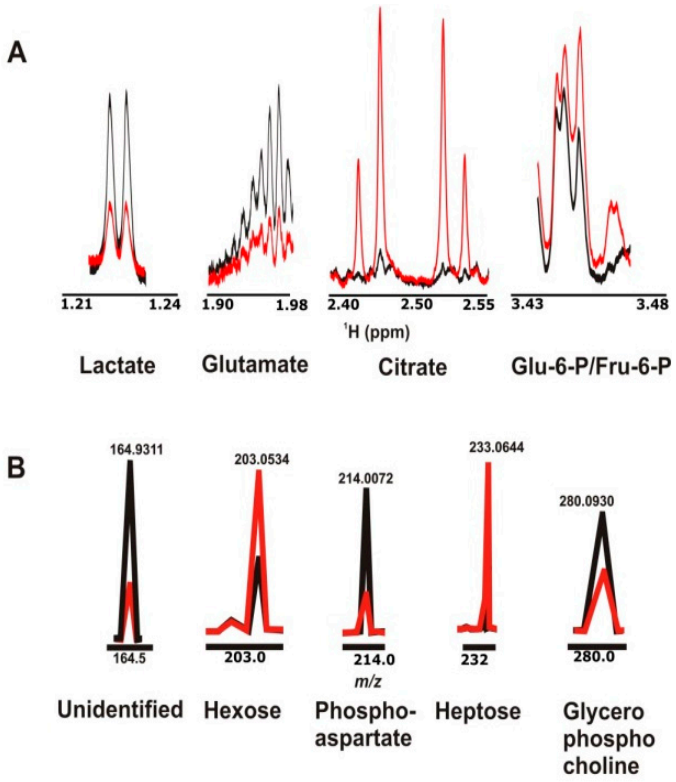
Cells Recover when Treated with Other Toxins



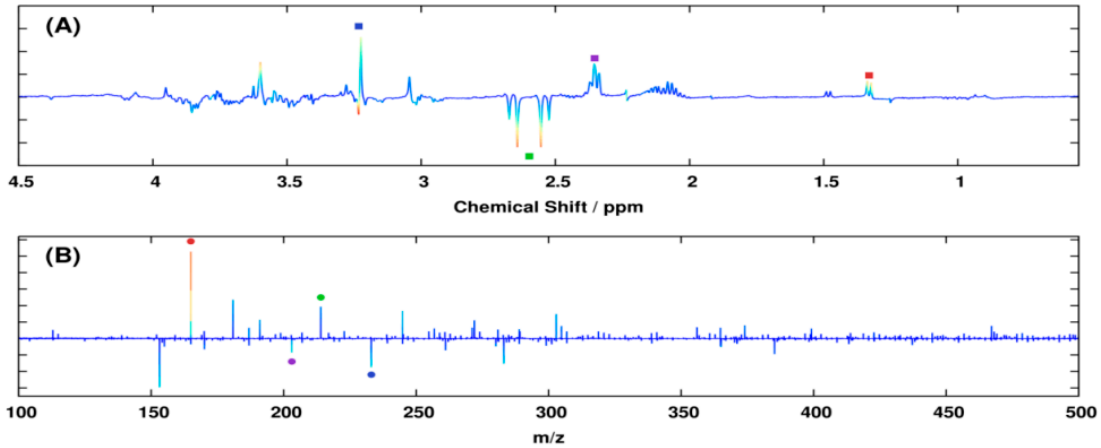
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

Results and discussion – Paraquat (PQ) Induces Dramatic Changes in metabolome of Dopaminergic Neuronal Cells

Integrate Data From Multiple Analytical Methods



NMR (A) and MS (B) paraquat-induced spectral changes

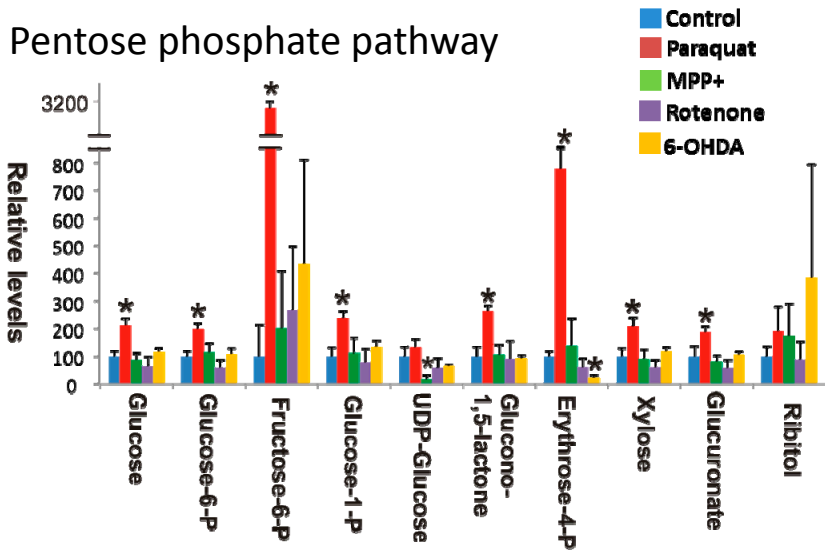


Backscaled multiblock-PLS-DA loadings from NMR (A) and MS (B) data

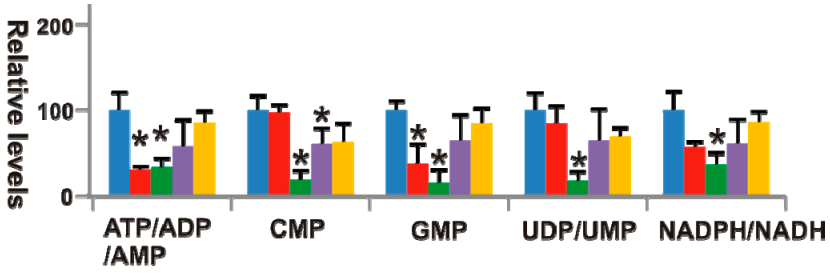
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

Results and discussion – Paraquat (PQ) Induces Dramatic Changes in Metabolome of Dopaminergic Neuronal Cells

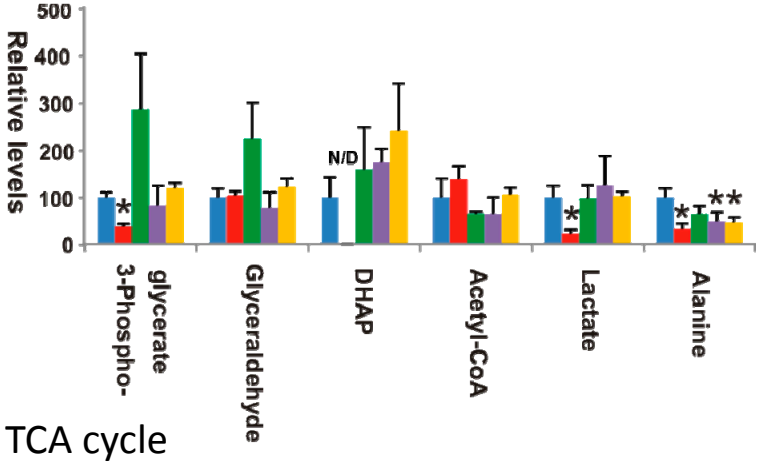
Pentose phosphate pathway



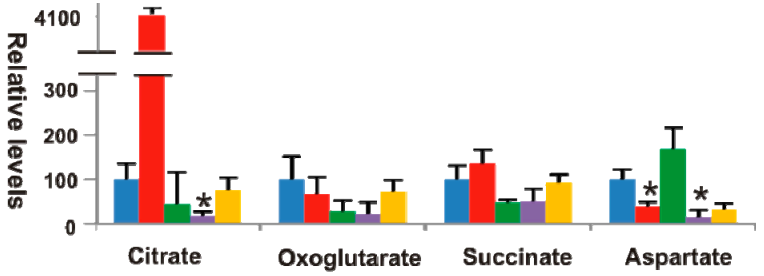
Nucleotide biosynthesis



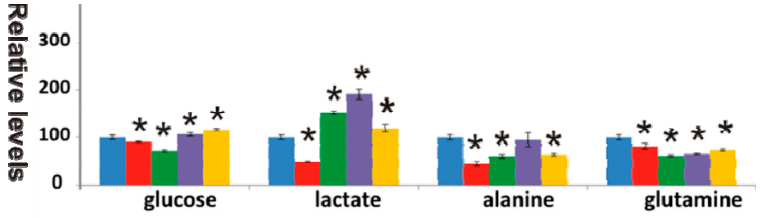
Glycolysis



TCA cycle



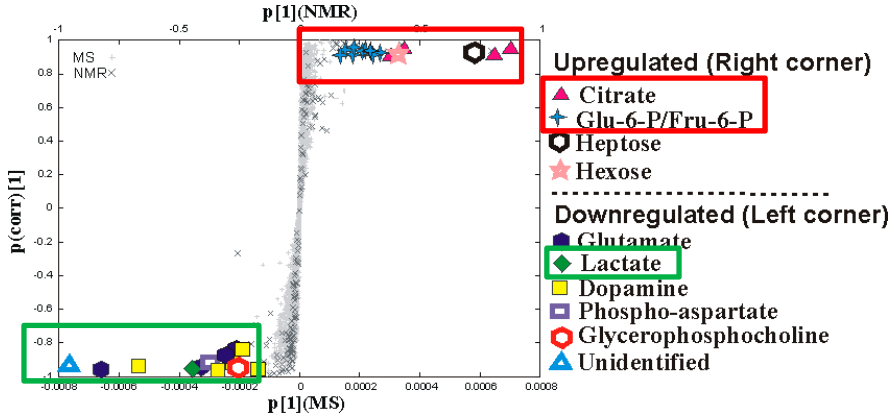
Glucose metabolism/extracellular media



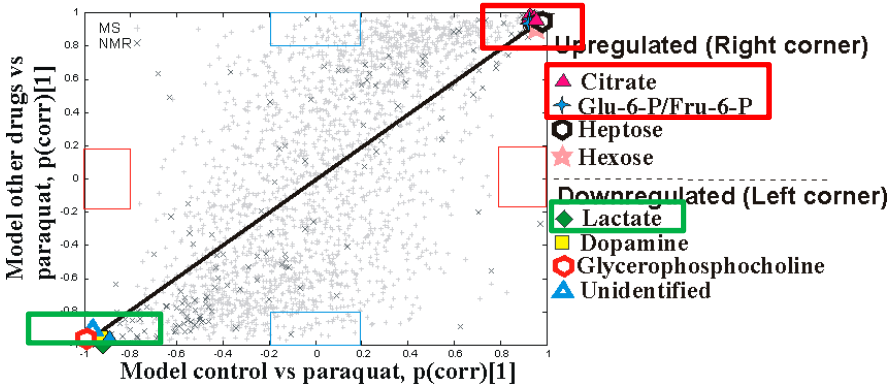
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

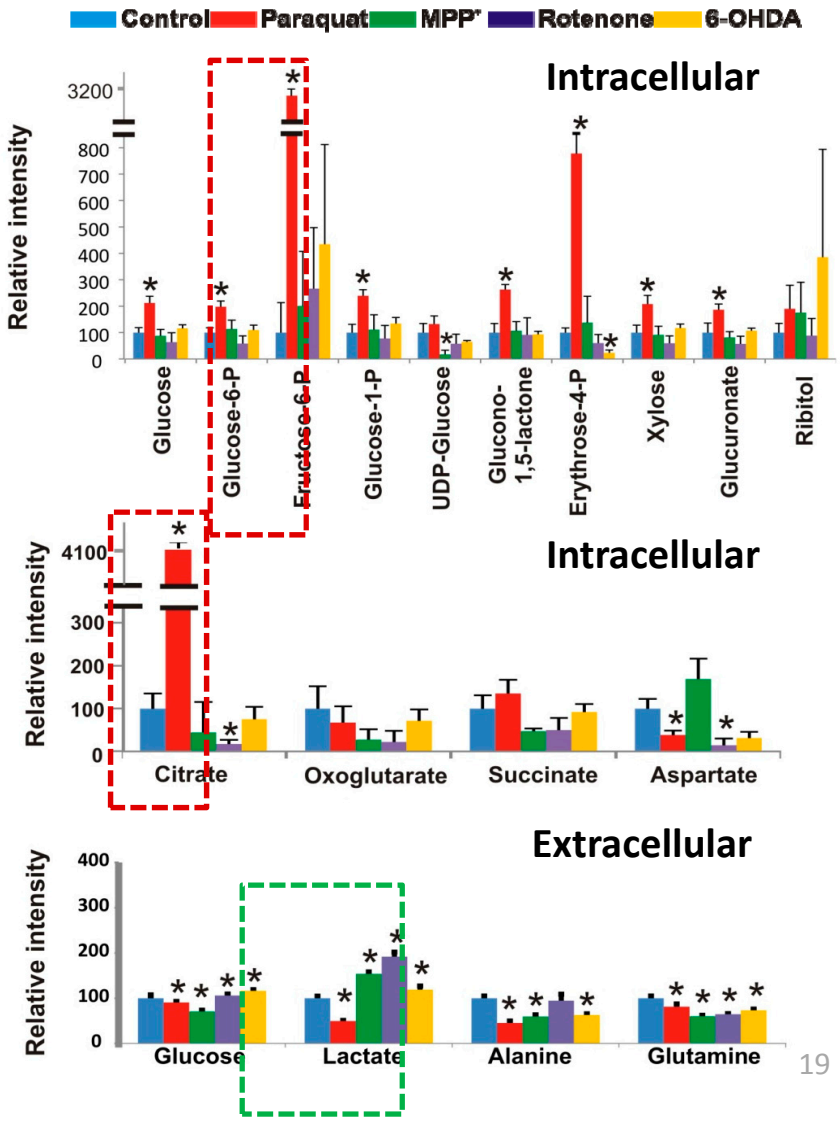
Results and discussion – Paraquat (PQ) Induces Alterations in Glucose Metabolism and Pentose Phosphate Pathway (PPP)

Paraquat vs control



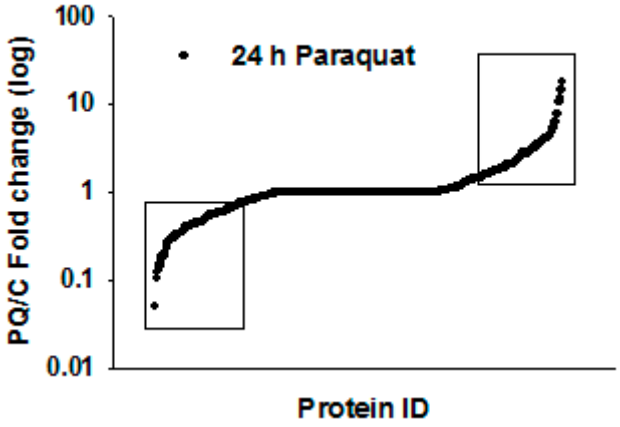
Paraquat vs other drugs

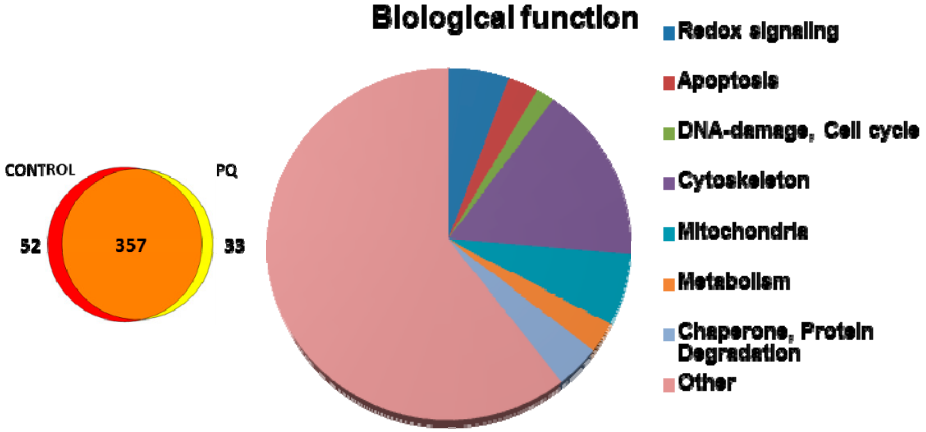


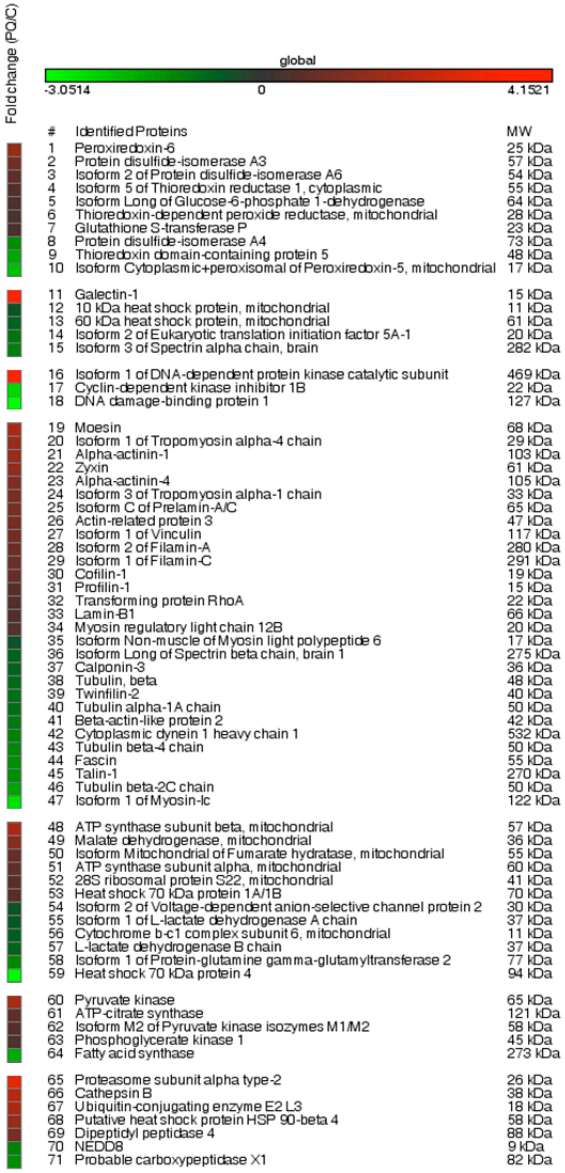


Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

Results and discussion – Paraquat (PQ) Induces Dramatic Changes in Proteome of Dopaminergic Neuronal Cells



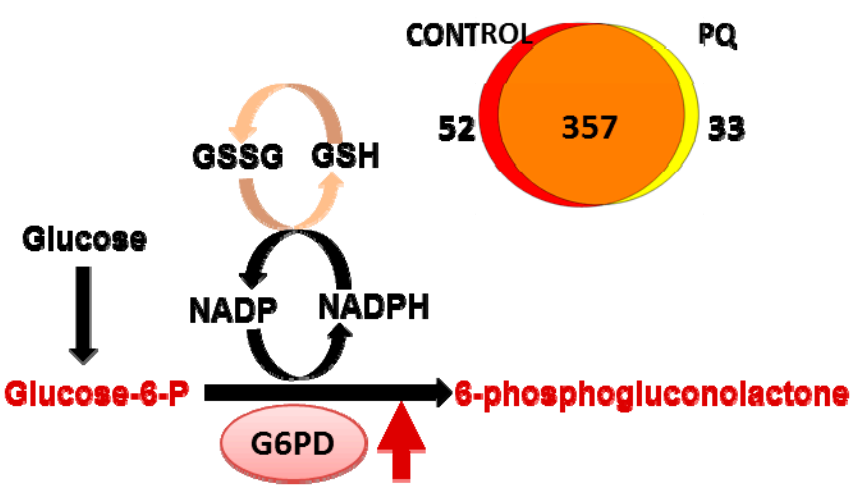


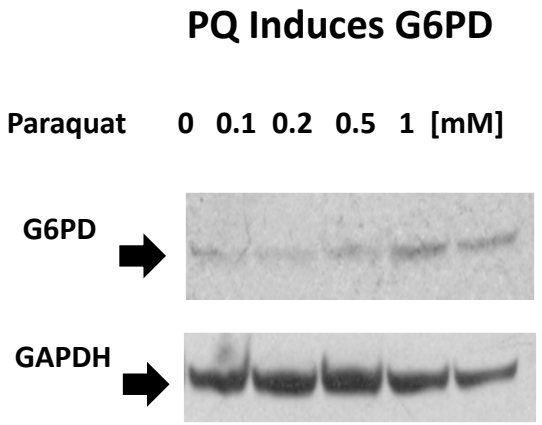


Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

Results and discussion – Integration of Metabolomics and Proteomics Data

- Increase in pentose phosphate pathway (PPP) enzymes
  - G6PD, glucose-6-phosphate dehydrogenase
- Increase in PPP metabolites
  - glucose 6-phosphate, fructose 6-phosphate, glucono-1,5-lactone and erythrose 4-phosphate
- Decrease in glycolysis and TCA cycle

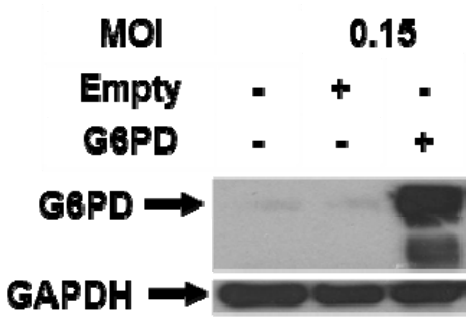


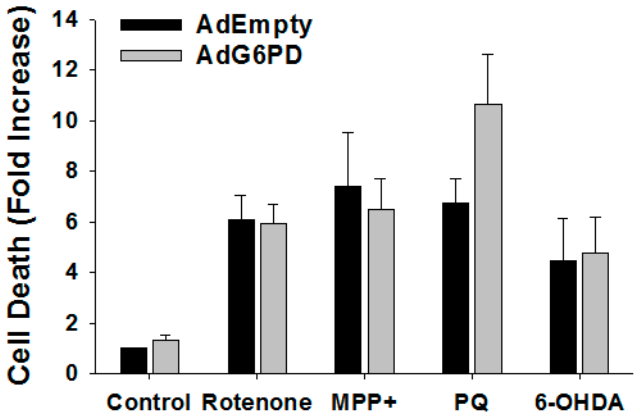


Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

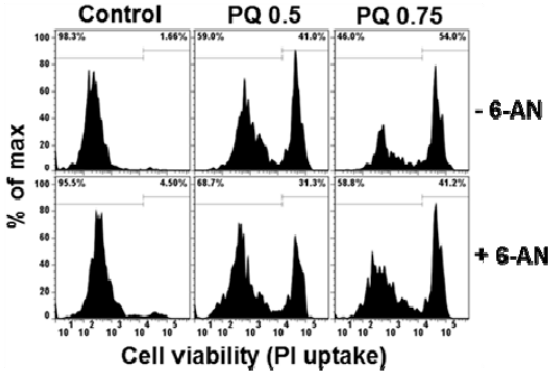
Results and discussion – G6PD Regulates Paraquat Toxicity

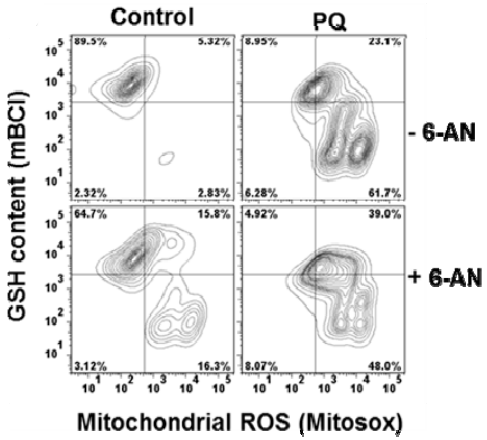
- Over-expression leads to increase in cell death with paraquat treatment G6PD
  - No change for other Mitochondrial/Environmental Toxins

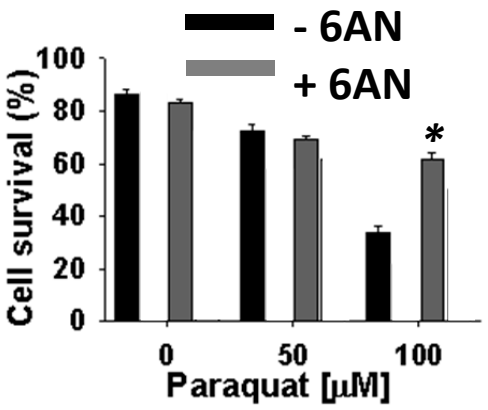




- Cell death and oxidative stress induced by PQ is alleviated by G6PD inhibitor
  - 6-AN, 6-aminonicotinamide



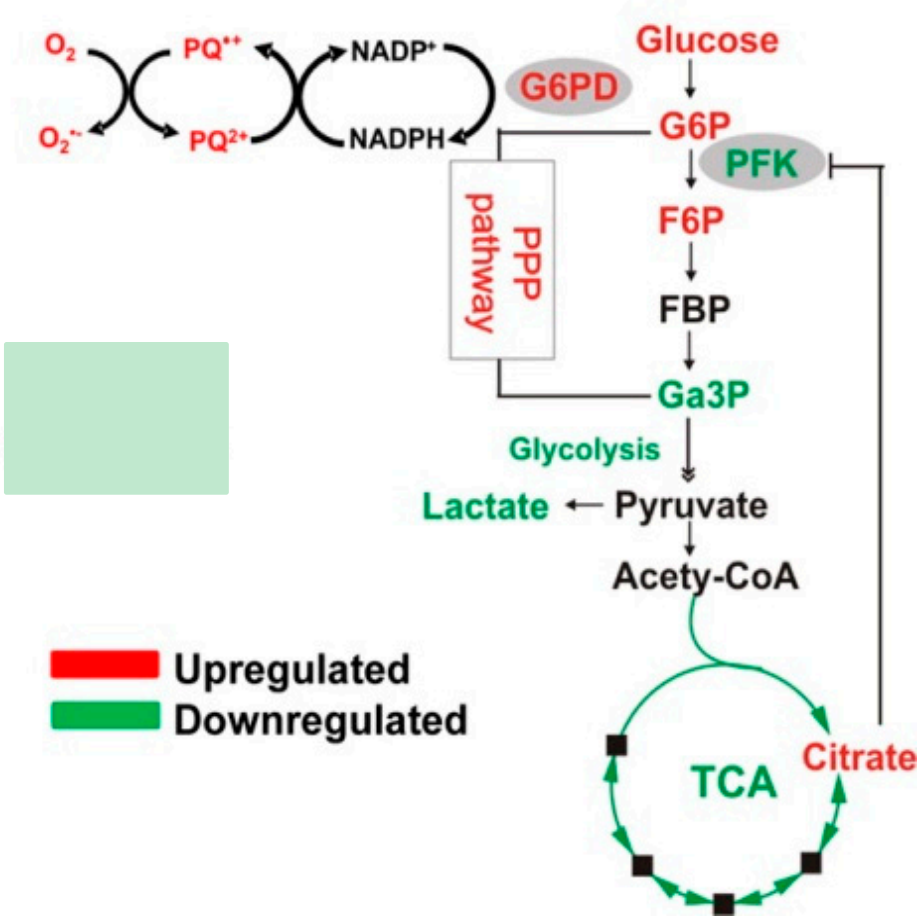




Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

Results and discussion – Paraquat "Hijacks" the Pentose Phosphate Pathway

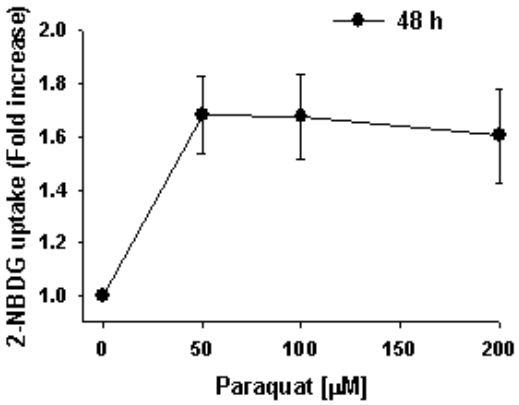
- **Paraquat-induced oxidative stress requires NADPH as an electron donor for its redox recycling**
  - **increases NADPH reducing equivalents**
  - **Stimulates paraquat redox cycling, oxidative stress and cell death**



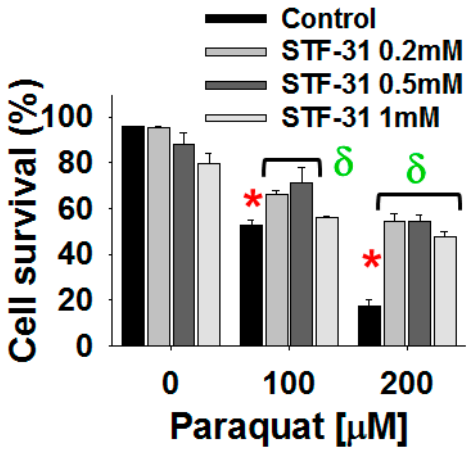
Anandham *et al.* (2016) *Mol. Neurobiol.* doi:10.1007/s12035-016-9906-2
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

Results and discussion – Glucose Metabolism Regulates PQ Toxicity

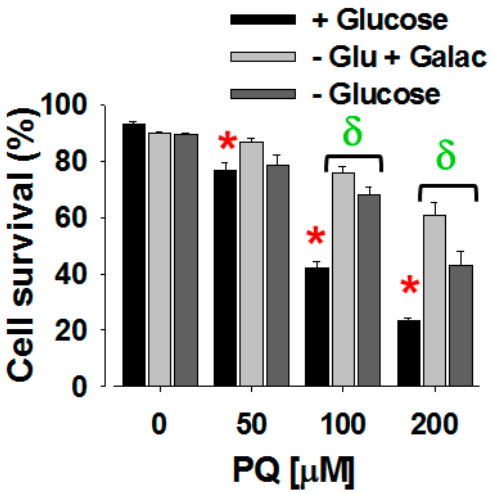
PQ Induces Glucose Uptake

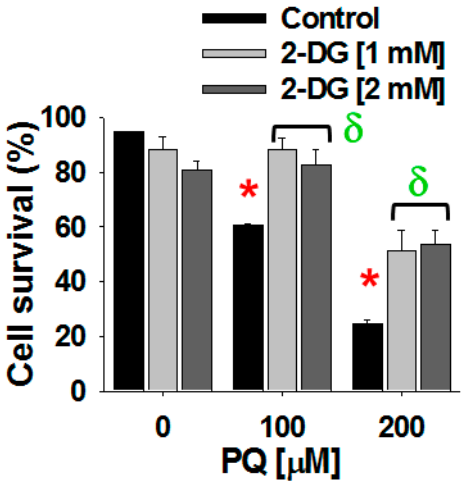


PQ Toxicity is Diminished with Inhibition of Glucose Transporter



PQ Toxicity is Diminished with Glucose Deprivation

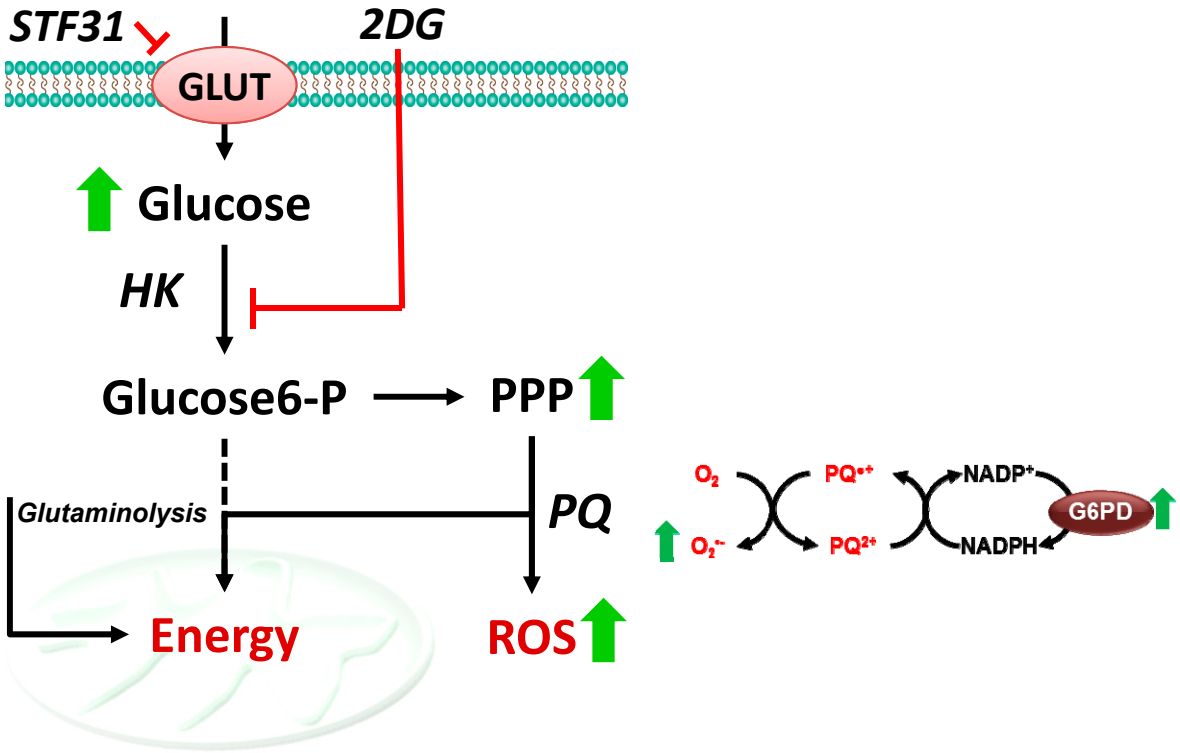




PQ Toxicity is Diminished with Inhibition of production of glucos-6-phosphate

Anandham *et al.* (2016) *Mol. Neurobiol.* doi:10.1007/s12035-016-9906-2
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

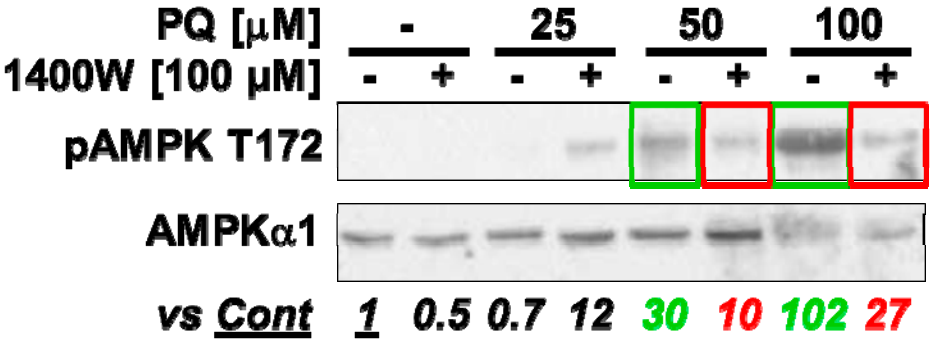
Results and discussion – Glucose Metabolism Regulates PQ Toxicity



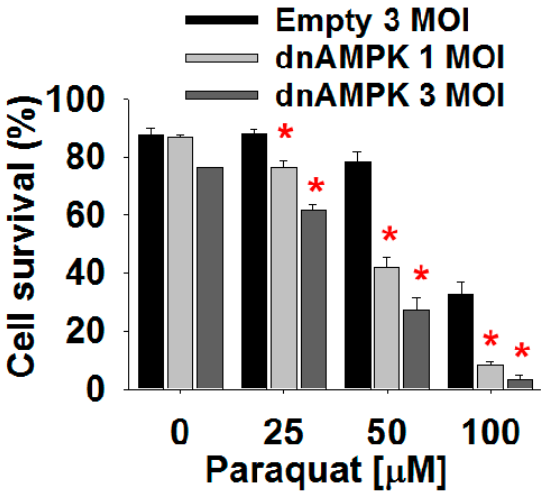
Anandham *et al.* (2016) *Mol. Neurobiol.* doi:10.1007/s12035-016-9906-2
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

Results and discussion – AMPK Protects Against PQ Toxicity

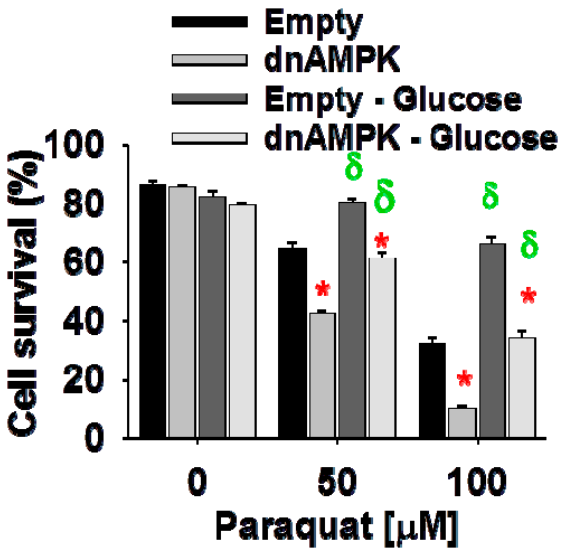
PQ Induces AMPK phosphorylation and activation



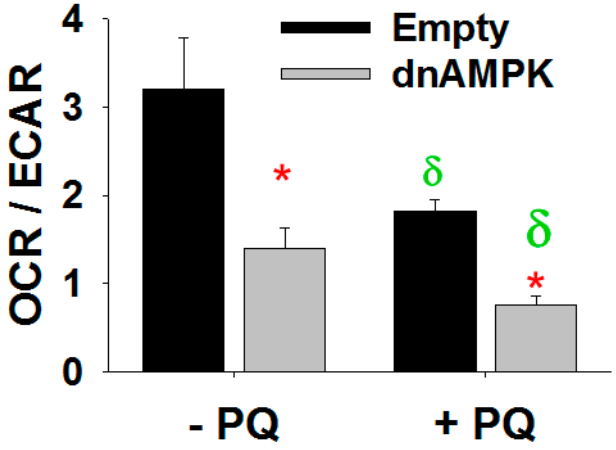
PQ Toxicity is Increased with Inhibition of AMPK



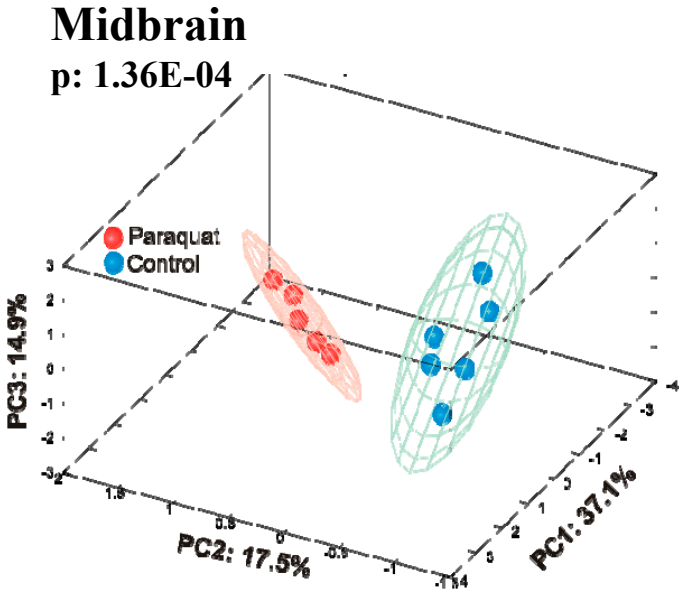
PQ Toxicity Increase due to AMPK inhibition is reversed with glucose deprivation

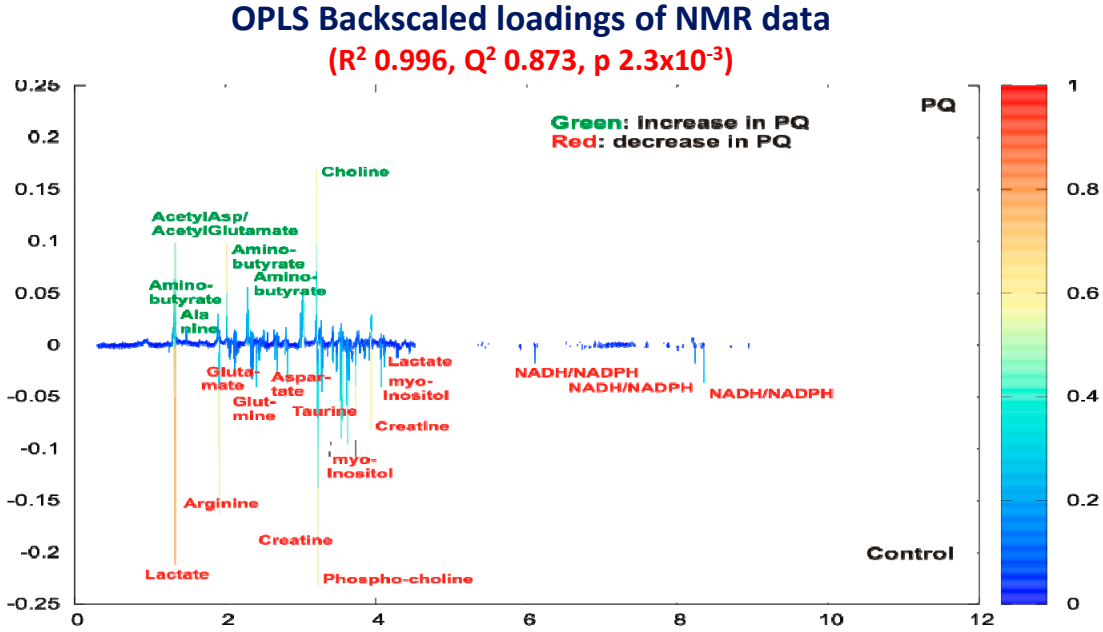


PQ & AMPK inhibition impairs glycolysis and mitochondrial respiration



Results and discussion –*In vivo* Metabolic Dysfunction Induced by PQ





- Paraquat-induced toxicity is brain region specific (no noticeable animal response)
- Largest impact on Midbrain – location of *substantia nigra*, where dopaminergic neurons are concentrated
  - Male C57/BL/6 mice (8-10 weeks old)
  - One intraperitoneal injection of 10 mg/kg paraquat or saline control twice a week for 9-weeks

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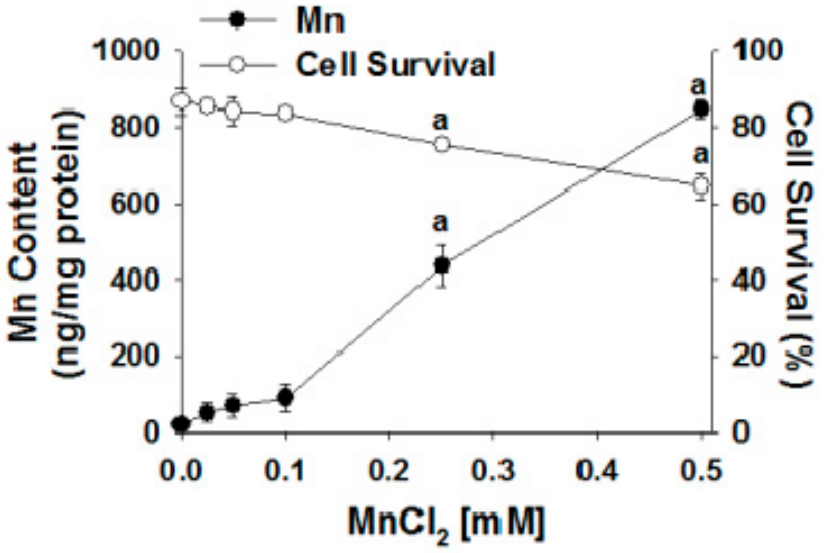
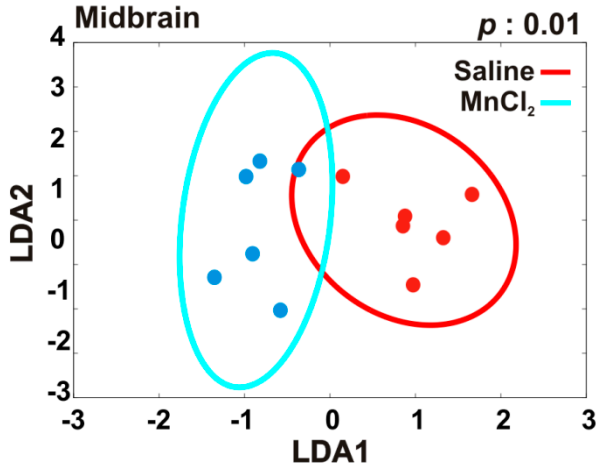
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285
Marshall *et al.* (2015) *Metabolomics*, 11:391-401

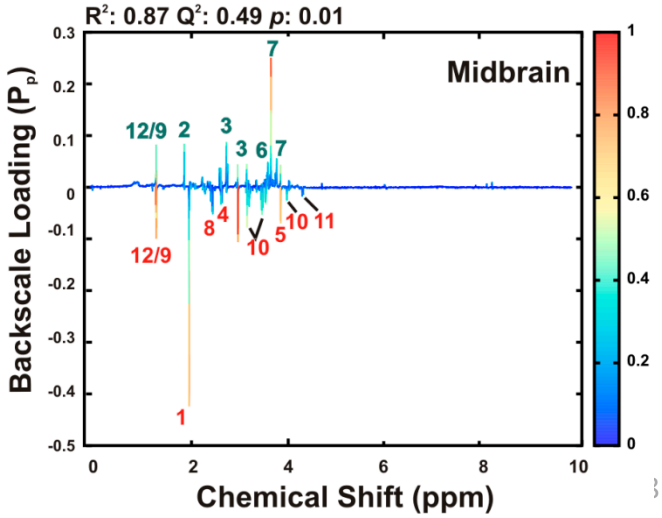
Results and discussion – Cell Death and Metabolic Dysfunction Induced by Manganese Toxicity

Mn Induced Cell Death Correlated with *in vivo* [Mn]



Mn Treatment Perturbs the Metabolome of the Midbrain

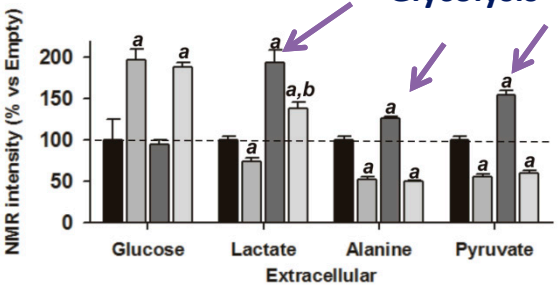




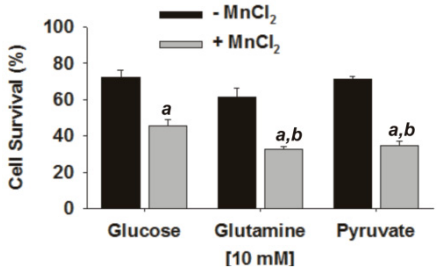
S. Lei *et al.* (2016) *J. Neurochem*, in preparation

Results and discussion – Mn Increases Glucose Metabolism and Malate-Aspartate Shuttle, and Decreases TCA Cycle

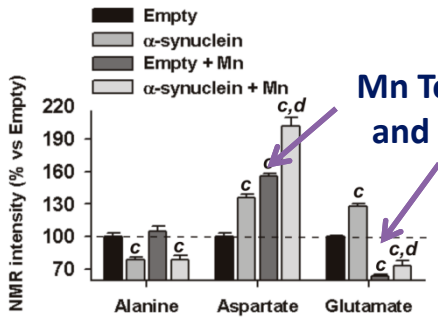
Mn Toxicity Increases Glycolysis



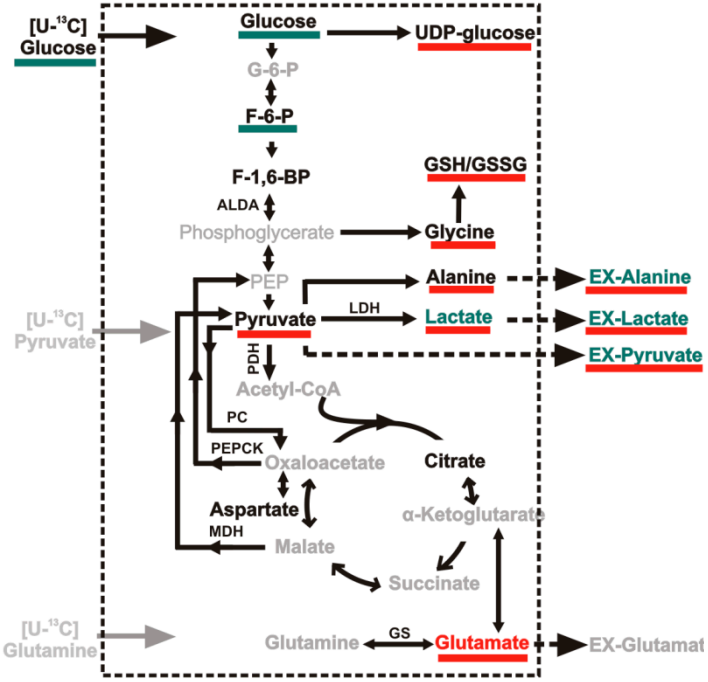
Mn Toxicity Increased Due to Glucose Deprivation



Mn Toxicity Increases MAS and Decreases TCA Cycle



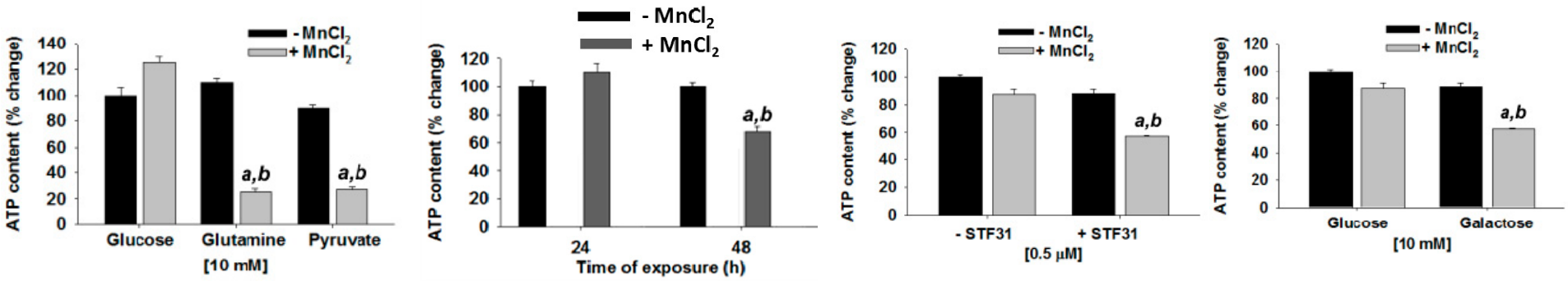
Mn Toxicity Leads to Impairment in Pyruvate Metabolism



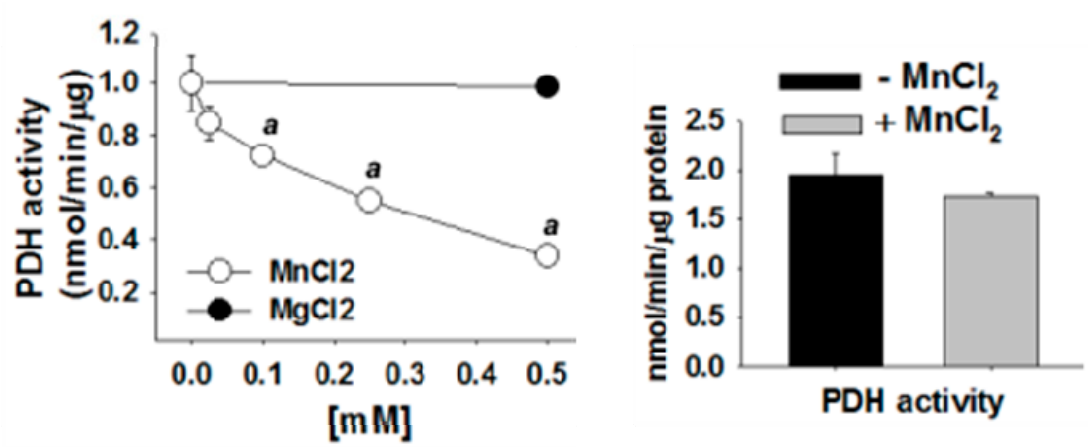
S. Lei *et al.* (2016) *J. Neurochem*, in preparation

Results and discussion – Mn Increases Glucose Metabolism and Malate-Aspartate Shuttle, and Decreases TCA Cycle

- **Mn toxicity produces an energy depletion**
  - **Inhibition of glycolysis enhances loss of ATP**



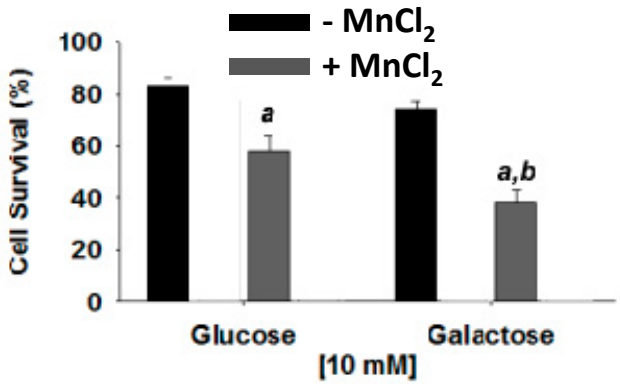
- **Mn appears to inhibit pyruvate dehydrogenase (PDH) activity**



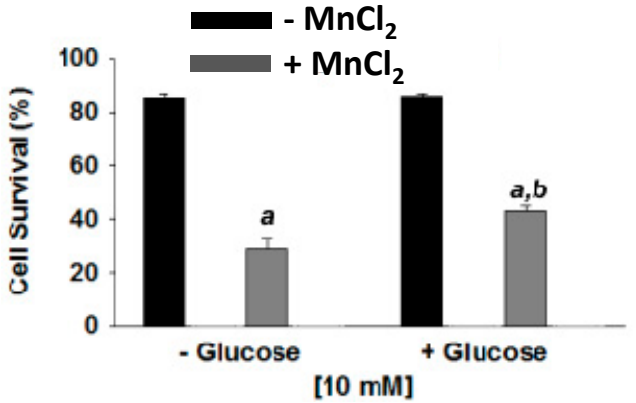
S. Lei *et al.* (2016) *J. Neurochem*, in preparation

Results and discussion – Impaired Glycolysis Enhances Mn Toxicity

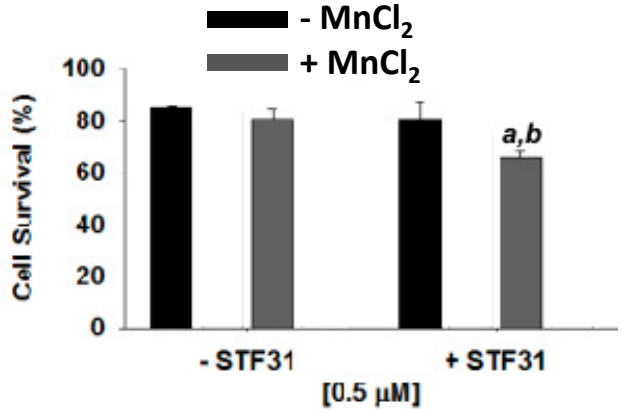
Mn Toxicity Increased Due to Replacing Glucose as Carbon Source



Mn Toxicity Increased Due to Glucose Deprivation

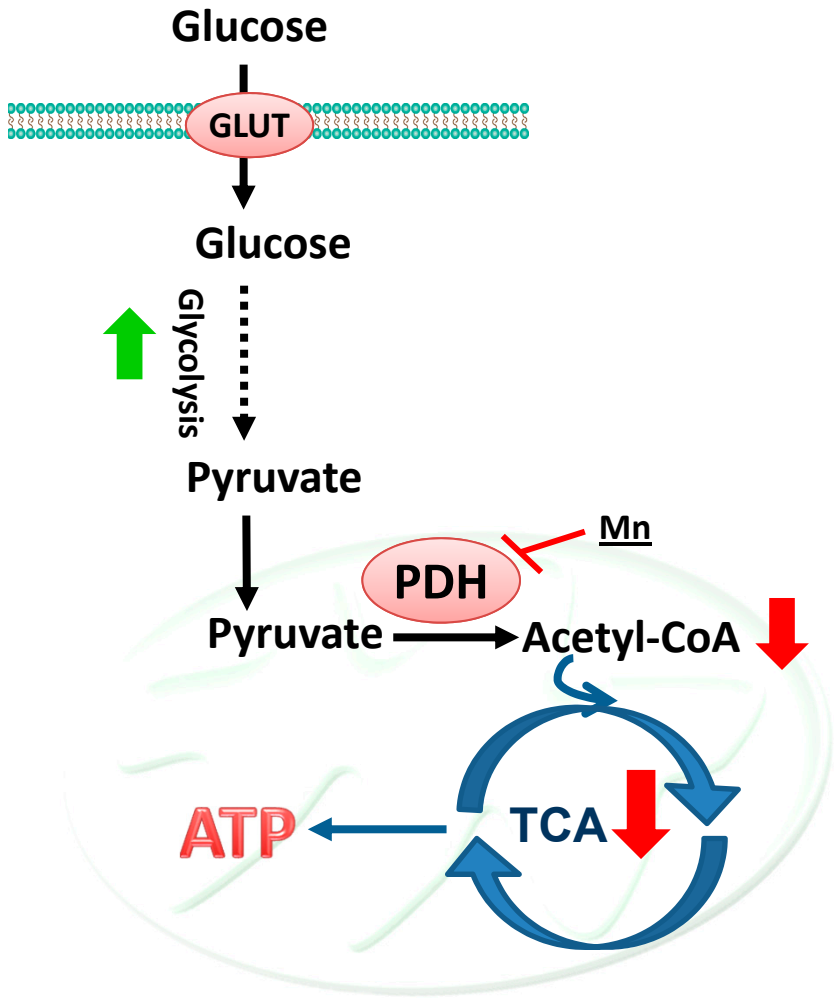


Mn Toxicity Increased Due to Decrease in Glucose Uptake



S. Lei *et al.* (2016) *J. Neurochem*, in preparation

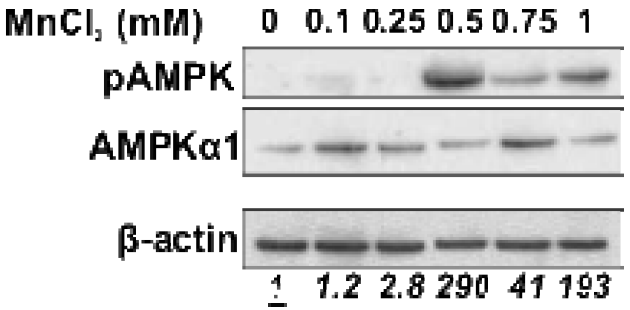
Results and discussion – Upregulated Glycolysis is the Metabolic Response to Energy Depletion Induced by Mn

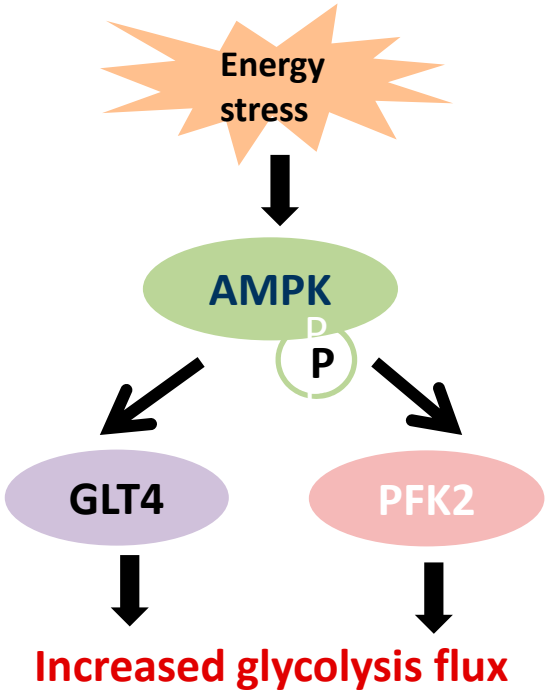


S. Lei *et al.* (2016) *J. Neurochem*, in preparation

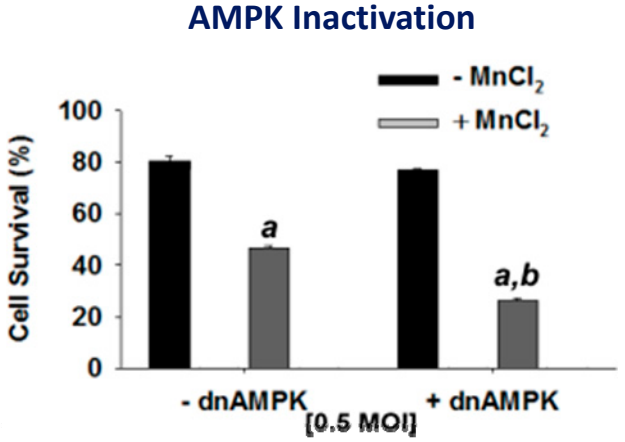
Results and discussion – AMPK Protects Against Mn Toxicity

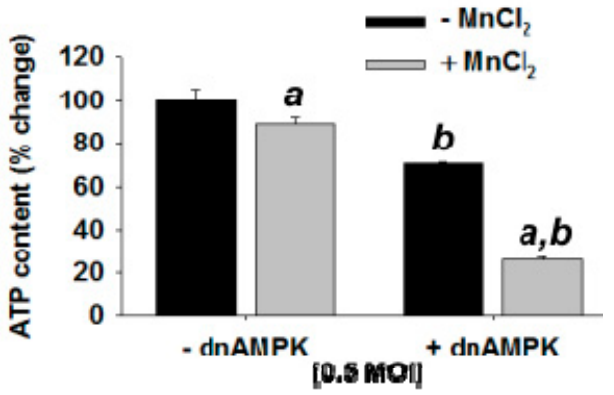
Mn Induces AMPK phosphorylation and activation





Mn Toxicity Increased Due AMPK Inactivation

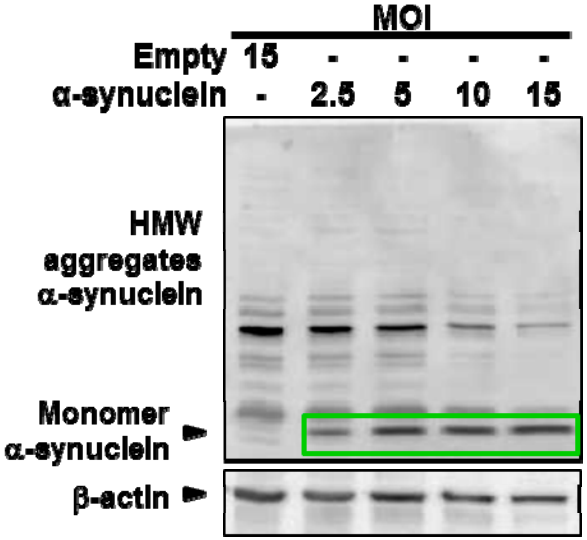




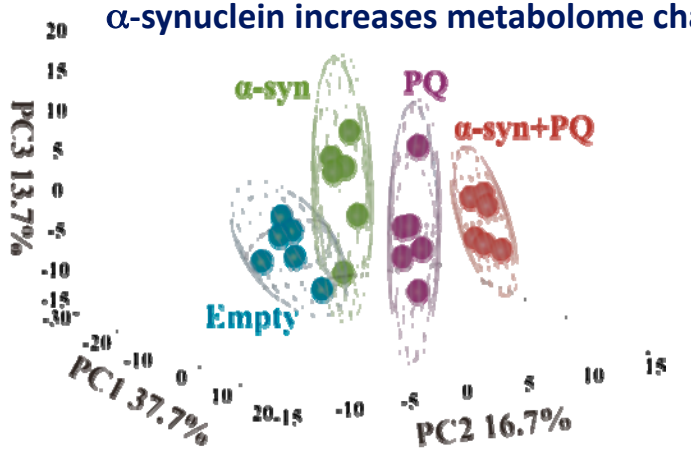
S. Lei *et al.* (2016) *J. Neurochem*, in preparation

Results and discussion – α-synuclein Potentiates PQ or Mn Toxicity and Metabolic Dysfunction

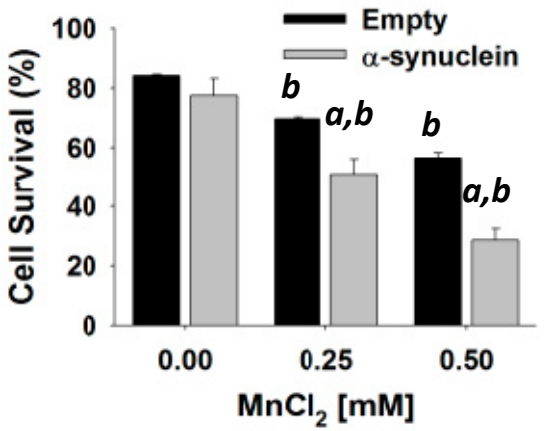
α-synuclein over-expression leads to high-MW aggregates

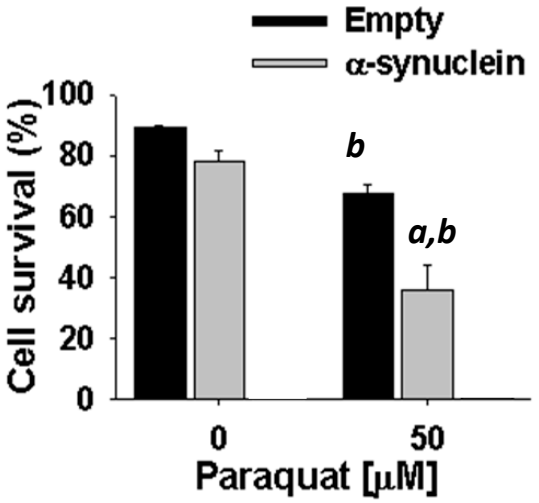


α-synuclein increases metabolome changes



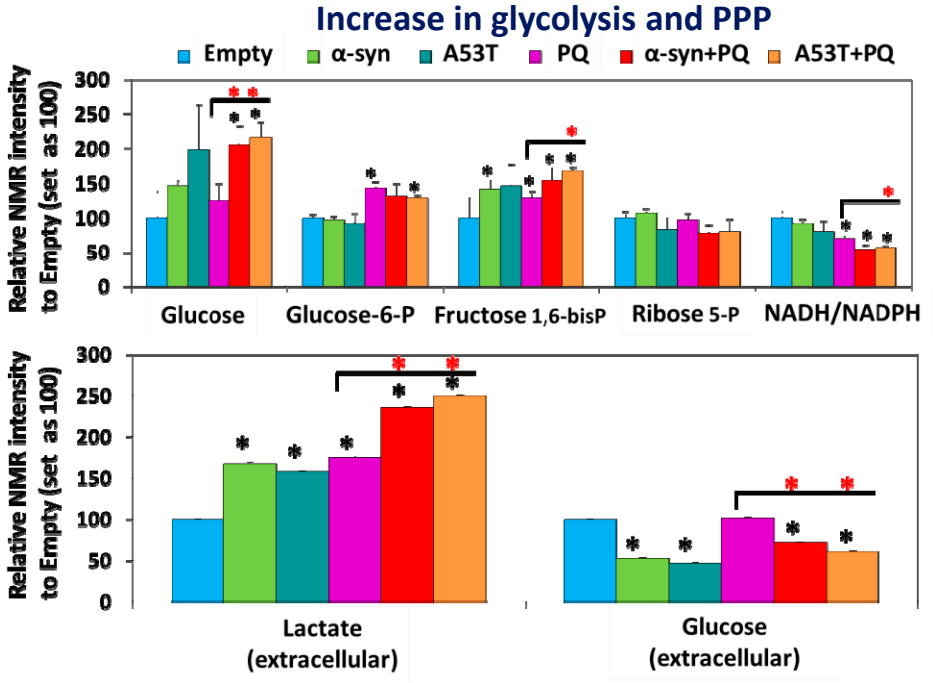
α-synuclein increase cell death

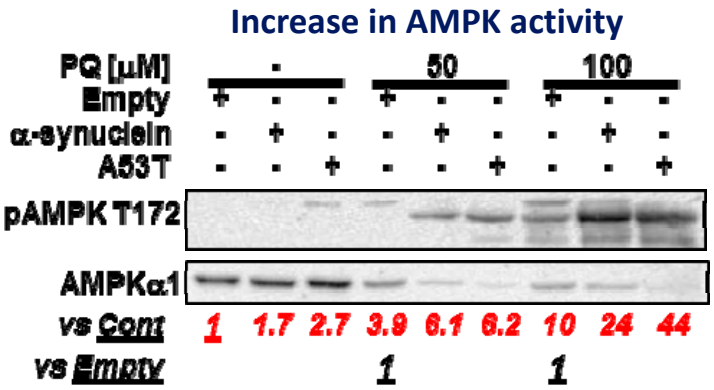


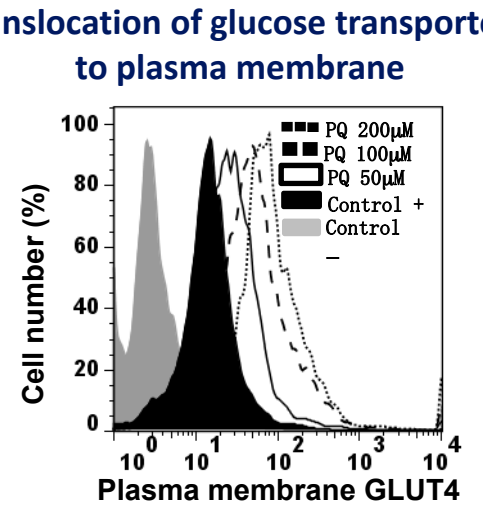


S. Lei *et al.* (2016) *J. Neurochem*, in preparation
Anandham *et al.* (2016) *Mol. Neurobiol.* doi:10.1007/s12035-016-9906-2
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285

Results and discussion – Glucose Metabolism Contributes to Synergistic Toxicity Between Paraquat and α-Synuclein







- **Overexpression in α-synuclein and exposure to paraquat:**
  - increase glucose uptake, glycolysis
  - translocation of glucose transporters to plasma membrane
  - upregulation of the pentose phosphate pathway
  - stimulated the activation of adenosine monophosphate-activated protein kinase (AMPK)
    - master regulator of metabolism in response to energy deficiency

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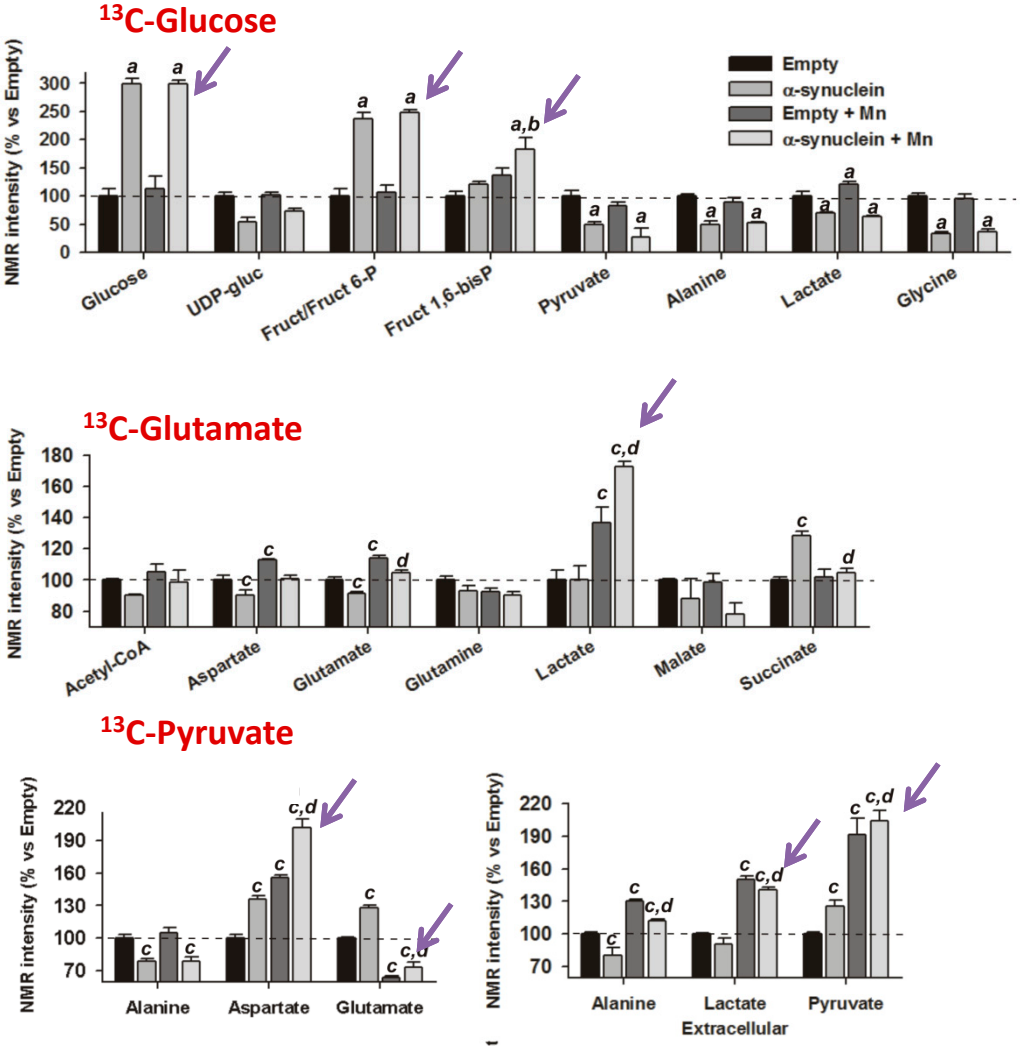
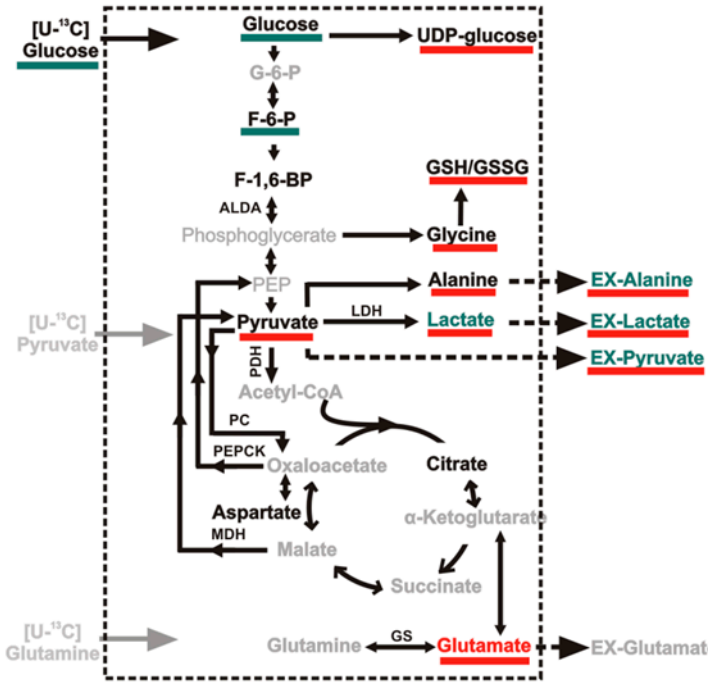
Results and discussion – Glucose Metabolism Contributes to Synergistic Toxicity Between Mn and α-Synuclein

α-synuclein Impairs Glycolysis and Negates Cell Response to Mn Toxicity





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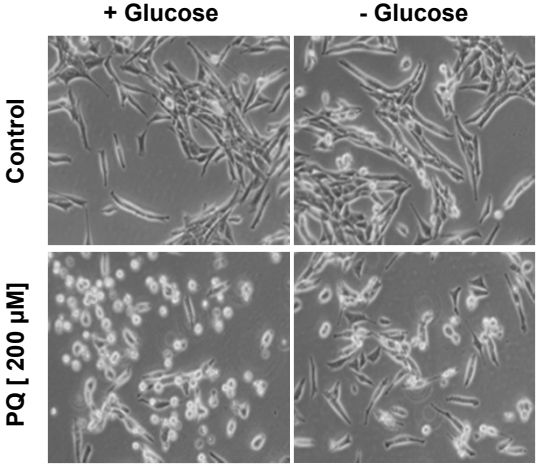
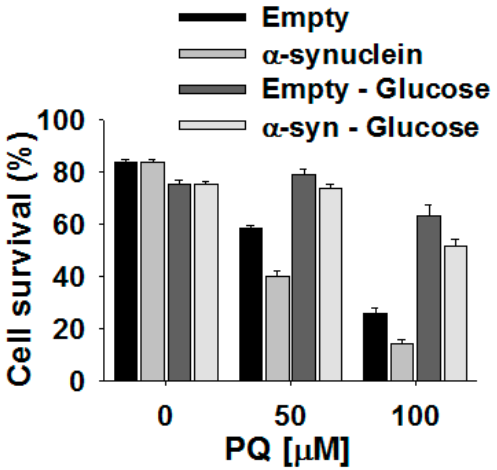
Results and discussion – Glucose Metabolism Contributes to Synergistic Toxicity Between PQ and α-Synuclein

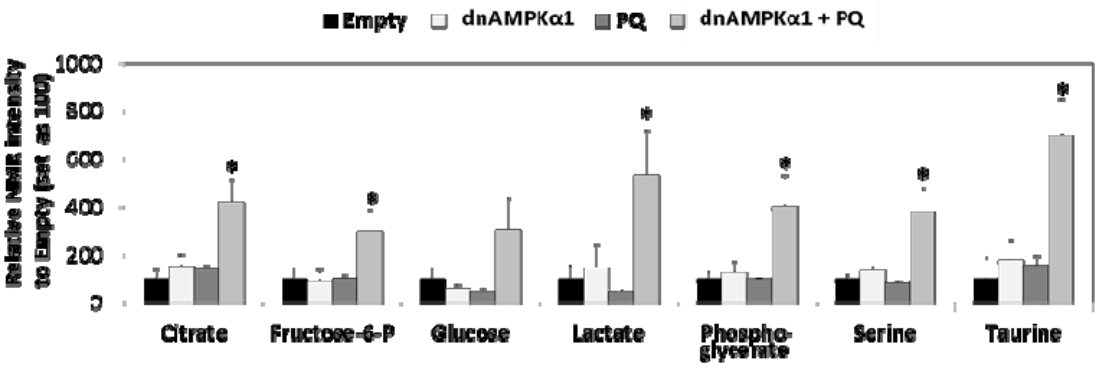
Glucose deprivation prevents paraquat induced cell death



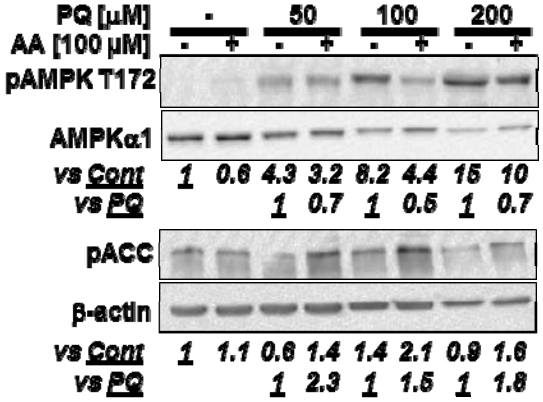


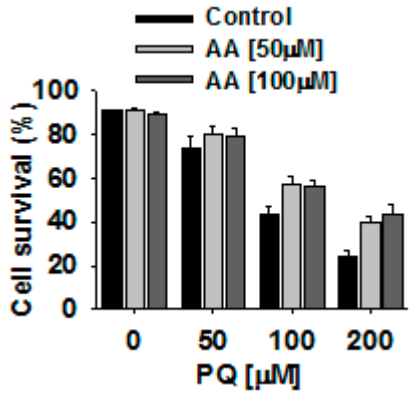
AMPK activation prevents paraquat induced cell death

(significant metabolic changes only observed with a dominant-negative form of AMPK)



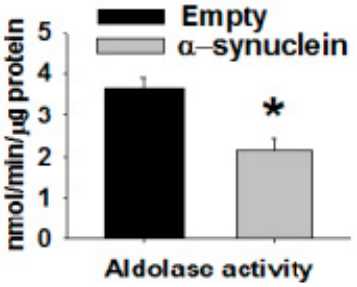
Ascorbic acid (AA) enhances AMPK activation prevents paraquat induced cell death

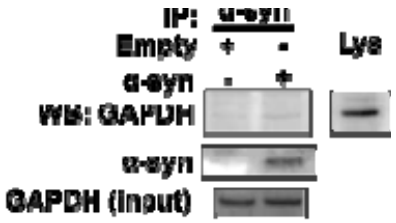


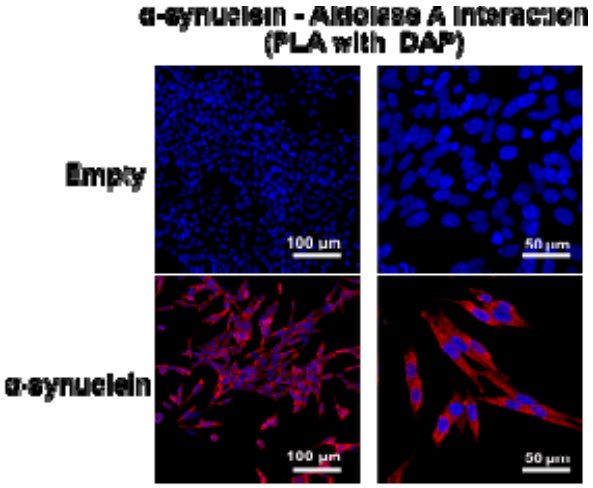


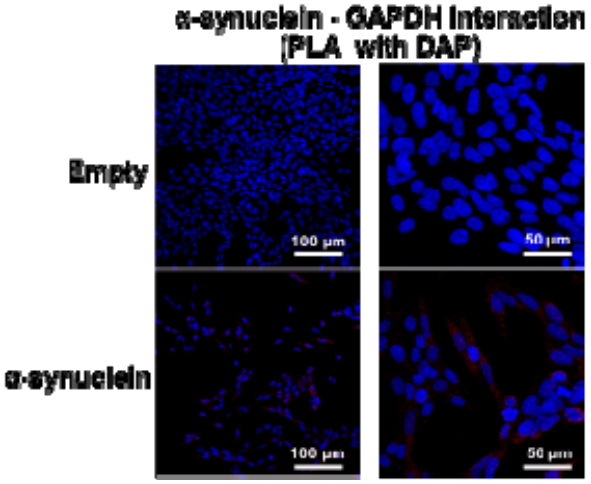
Anandhan A. , *et al Molecular neurobiology* (2016), doi:10.1007/s12035-016-9906-2

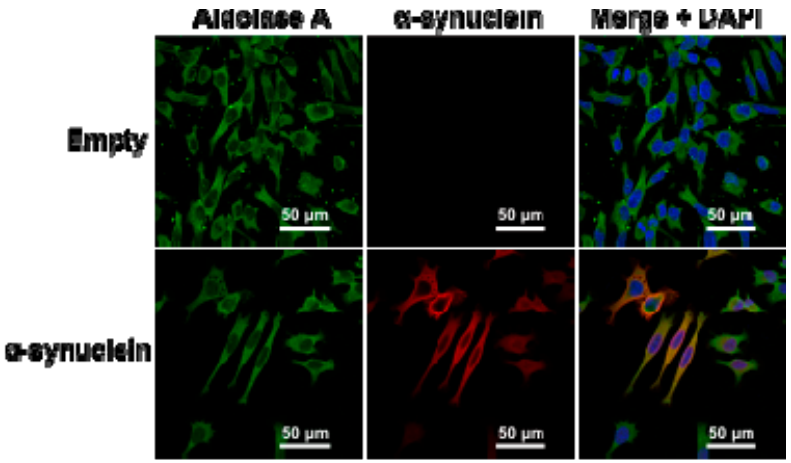
Results and discussion – α-synuclein Impairs Aldolase Activity Through a Protein-Protein Interaction

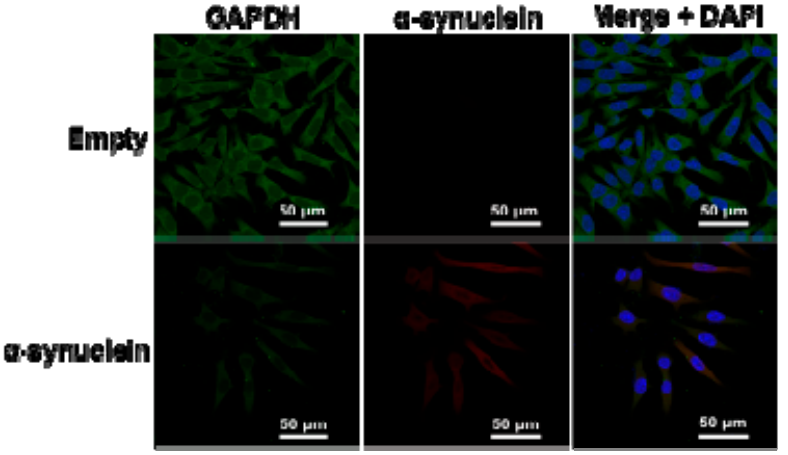






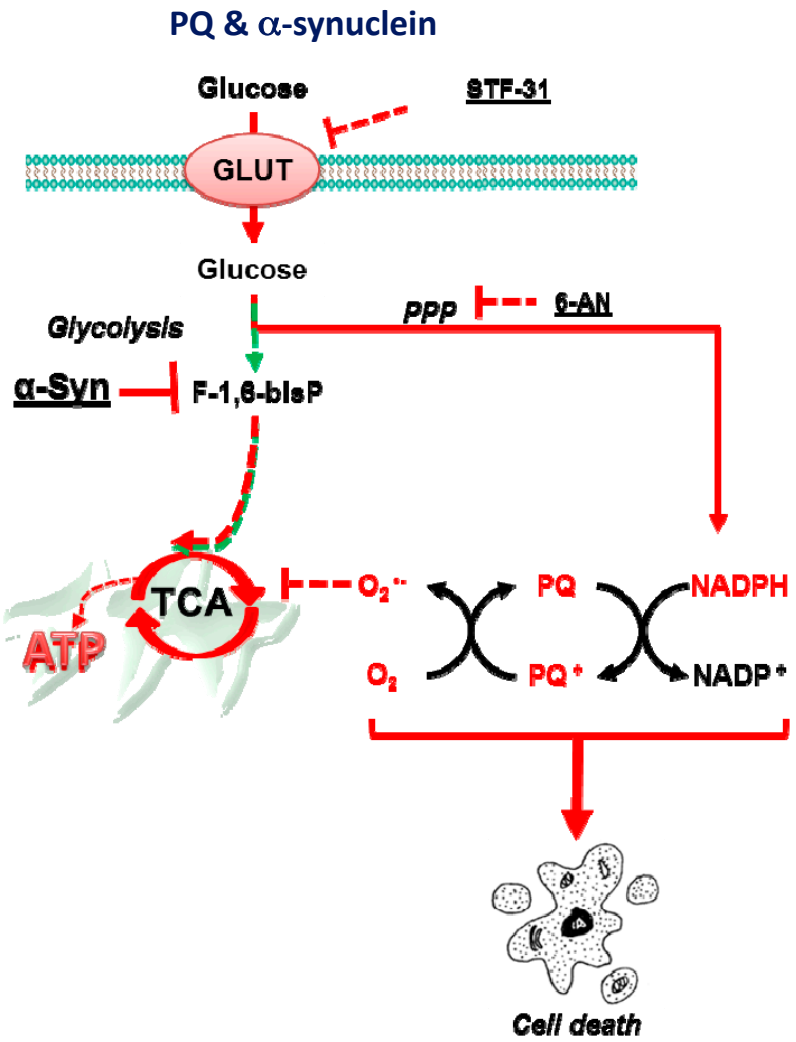


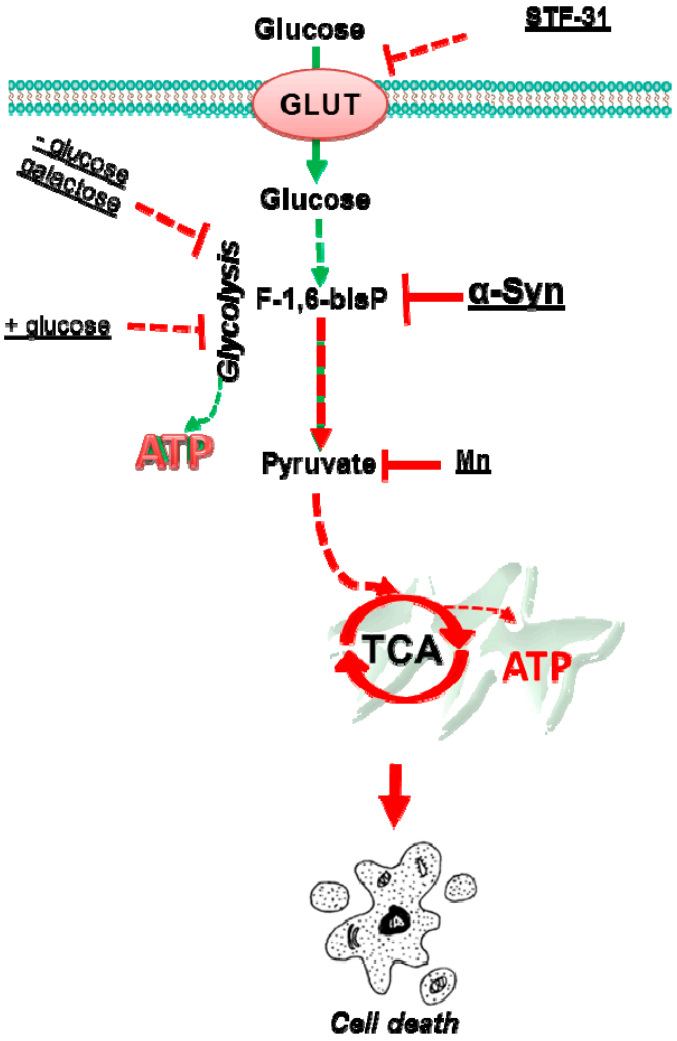




S. Lei *et al.* (2016) *J. Neurochem*, in preparation

Results and discussion – α-synuclein Potentiates PQ or Mn Toxicity and Metabolic Dysfunction





S. Lei *et al.* (2016) *J. Neurochem*, in preparation
Anandham *et al.* (2016) *Mol. Neurobiol.* doi:10.1007/s12035-016-9906-2
Lei *et al.* (2014) *ACS Chem Biol.* 9(2):282-285

Conclusions

- **Paraquat**
  - hijacks the NADPH from the PPP to redox cycle, induce oxidative damage and impair antioxidant defenses
  - increases glucose transport and carbon flux to the PPP.
  - Impairs TCA cycle leading to increased citrate accumulation, which leads to an impairment in glycolysis
- **Manganese**
  - Toxicity results in energy depletion
  - Inhibits pyruvate dehydrogenase
  - Induces an increase in glycolysis
- **α-synuclein**
  - Inhibits Aldolase activity
  - Impairs glycolysis and upregulates glucose transport
    - Channels carbon flux to the PPP to increase PQ's redox cycling and ROS formation
  - Potentiates environmental toxicity (Manganese and Paraquat)
    - Facilitates ATP depletion induced by Mn exposure
- **Glucose metabolism regulates α-synuclein + PQ toxicity**
  - Paraquat increases glucose transport and translocation of glucose transporters.
  - Inhibition of GLUT-like transporters prevents α-synuclein + PQ toxicity.
  - Inhibition of PPP protects against α-synuclein + PQ
- **AMPK signaling exerts a protective effect**
  - Activation of AMPK can be mediated by both ROS and ATP depletion.

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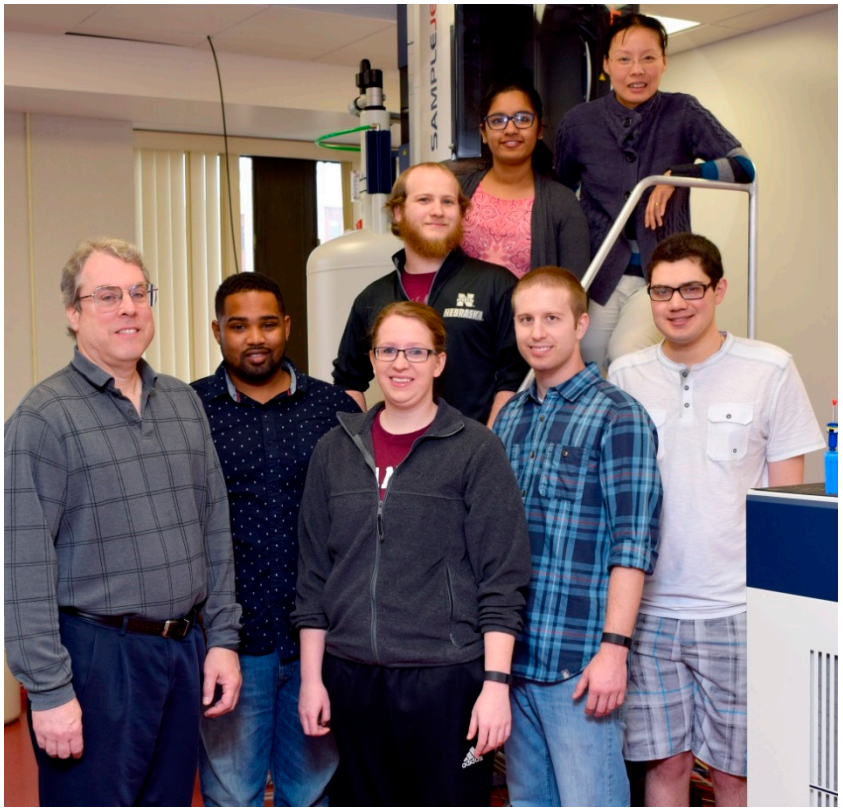
Acknowledgments



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Front Row: Dr. Robert Powers, Darrell Marshall, Tessa Andrews, Jonathan Catazaro, Lukas Brenden; Not Pictured: Teklab Gebregiworgis, Samantha Lonergan, AJ Lowe, Brad Worley, Bo Zhang, Steve Halouska

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